

## Supplemental Tables

**Table S1, related to Figures 1, 6 and 7. RNA-seq sample information.**

Sample	Raw read pairs	Trimmed read pairs (125 bp) for STAR input	STAR unique mapping rate	Trimmed read pairs for Salmon input	Salmon mapping rate
WT_1	34222771	27870420	86.71	33922569	90.37
WT_2	39737843	32252735	86.49	39393855	89.68
WT_3	38588717	34454350	87.45	38228994	88.28
MUT_1	34129241	29490238	87.67	33845444	90.14
MUT_2	36883110	31631761	87.66	36506989	88.51
MUT_3	42198499	36007926	87.27	41818779	89.45
Control_1	41471062	28063811	88.15	41450695	90.67
Control_2	42396709	29548001	87.39	42352348	90.87
Control_3	53748421	37788049	87.23	53718575	89.79
cKO_1	48542139	34411636	87.29	48519668	89.05
cKO_2	70099132	48039070	86.37	70051229	90.39
cKO_3	45248549	31400746	87.25	45211149	90.30

WT = *Pdgfra*<sup>+/+</sup>; MUT = *Pdgfra*<sup>PI3K/PI3K</sup>; Control = *Srsf3*<sup>fl/fl</sup>; *Wnt1-Cre*<sup>+/+</sup>; cKO = *Srsf3*<sup>fl/fl</sup>; *Wnt1-Cre*<sup>+Tg</sup>

**Table S3, related to Figure 1.** Gene name, exon coordinates and  $\Delta$ PSI values from *Pdgfra*<sup>+/+</sup> versus *Pdgfra*<sup>PI3K/PI3K</sup> RNA-seq analysis for skipped exon events with one or more Srsf3 motifs and a corresponding mouse model with a craniofacial phenotype. Mouse models with a cleft secondary palate are highlighted in bold.

<b>Gene</b>	<b>Exon start</b>	<b>Exon end</b>	<b><math>\Delta</math>PSI</b>
<i>Gng8</i>	chr7:16893120	chr7:16893219	36.1
<i>Vdr</i>	chr15:97869317	chr15:97869423	25.4
<i>Gng8</i>	chr7:16892449	chr7:16892511	20.8
<i>Akap10</i>	chr11:61904774	chr11:61904859	19.9
<i>Ptk2</i>	chr15:73365040	chr15:73365195	18.4
<i>Nf1</i>	chr11:79463232	chr11:79463295	18.0
<i>Bcas3</i>	chr11:85822021	chr11:85822112	17.5
<b><i>Fuz</i></b>	chr7:44896546	chr7:44896645	16.7
<b><i>Fuz</i></b>	chr7:44896546	chr7:44896668	15.4
<b><i>Chrd</i></b>	chr16:20739953	chr16:20740056	14.3
<b><i>Fuz</i></b>	chr7:44896914	chr7:44896999	13.1
<b><i>Cask</i></b>	chrX:13552372	chrX:13552441	11.3
<i>Vps54</i>	chr11:21268784	chr11:21268863	10.2
<b><i>Smad7</i></b>	chr18:75375873	chr18:75375948	9.5
<b><i>Qrich1</i></b>	chr9:108518766	chr9:108518826	8.2
<i>Sptan1</i>	chr2:30001099	chr2:30001159	7.8
<i>Dyrk1a</i>	chr16:94611418	chr16:94611504	7.7
<i>E2f5</i>	chr3:14602647	chr3:14602712	6.5
<i>Zeb2</i>	chr2:45017396	chr2:45017465	6.3
<b><i>Map3k7</i></b>	chr4:31994873	chr4:31994954	6.2
<i>Apaf1</i>	chr10:91023719	chr10:91023848	5.6
<b><i>Tgfb2</i></b>	chr1:186690720	chr1:186690804	5.2
<i>Inpp1l</i>	chr7:101823512	chr7:101823646	-5.7
<i>Pdpk1</i>	chr17:24107228	chr17:24107271	-12.6
<b><i>Dzip1l</i></b>	chr9:99632636	chr9:99632748	-14.4

**Table S5, related to Figure 4. Progeny from  $Srsf3^{fl/fl}$  x  $Srsf3^{fl/fl};Wnt1-Cre^{+/Tg}$  crosses.**

Age	$Srsf3^{+/fl}; W1C^{+/+}$	$Srsf3^{+/fl}; W1C^{+/Tg}$	$Srsf3^{fl/fl}; W1C^{+/+}$	$Srsf3^{fl/fl}; W1C^{+/Tg}$				Chi-square*
				Total	Live	Dead but developmentally equal	Resorbed/delayed	
E8.5	6	4	11	7	7 (25.0%)	0	0	p = 1.0000
E9.5	13	9	11	9	9 (21.4%)	0	0	p = 0.5930
E10.5	35	32	32	34	30 (22.6%)	1	3	p = 0.7613
E11.5	32	26	18	22	20 (20.4%)	1	1	p = 0.4460
E12.5	12	15	20	10	6 (10.5%)	1	3	p = 0.0411
E14.5	5	5	2	2	1 (7.1%)	0	1	p = 0.1495
E15.5	14	8	13	7	0 (0%)	0	7	p = 0.0006
E18.5	9	8	7	3	1 (3.7%)	2	0	p = 0.0956
P0	18	14	20	2	0 (0%)	2	0	p = 0.0003

\*Chi square analysis was performed with live and dead but developmentally equal counts for  $Srsf3^{fl/fl};Wnt1-Cre^{+/Tg}$  embryos

**Table S7, related to Figure 6.** Gene name and log<sub>2</sub>(fold change) values from *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+/+</sup>* versus *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+Tg</sup>* RNA-seq analysis for differentially-expressed genes with a corresponding mouse model with a craniofacial phenotype. Mouse models with a cleft secondary palate are highlighted in bold.

Gene	log <sub>2</sub> (fold change)
<b>Sim2</b>	4.15812767
<i>Ascl1</i>	3.90697996
<i>Emx2</i>	3.27243847
<i>Grem2</i>	2.99306483
<i>Thrb</i>	2.85205581
<i>Smoc2</i>	2.74134256
<b>Inhba</b>	2.52841522
<i>Aldh1a2</i>	2.42461884
<b>Kcnj2</b>	2.28766851
<i>Capn6</i>	1.39714305
<b>Eya4</b>	1.39646025
<i>Alx1</i>	1.17541114
<i>Slc8a1</i>	1.17422346
<i>L1cam</i>	1.16812077
<i>Eda</i>	1.12211529
<i>Lgals1</i>	0.99776335
<i>Gata3</i>	0.99406115
<b>Foxd3</b>	0.98552765
<b>Pcsk5</b>	0.81156592
<b>Fgf10</b>	0.80927165
<i>Alx4</i>	0.72872988
<i>Bmp1</i>	0.59722314
<i>Irx3</i>	0.58022535
<b>Gli2</b>	0.55774872
<i>Cdon</i>	0.54318469
<i>Sulf2</i>	0.51966198
<i>Lima1</i>	0.48945473
<b>Pdgfc</b>	-0.6367654
<b>Piga</b>	-0.6485028
<i>Col18a1</i>	-0.6782651
<i>Notch1</i>	-0.7699911
<i>Cdh5</i>	-0.7887263
<i>Etv5</i>	-0.7957678
<b>Col2a1</b>	-0.8217745
<i>Lyn</i>	-0.9220268
<i>Fbn2</i>	-0.9424215
<i>Klf2</i>	-0.9430508
<b>Hspg2</b>	-0.9611855
<b>Jag2</b>	-0.9686243
<i>Kdr</i>	-0.985219

<b>Fras1</b>	-1.0847412
<i>Cxxc5</i>	-1.2326119
<i>Tfpi</i>	-1.2517335
<i>Eng</i>	-1.2579656
<b>Tbx1</b>	-1.5287566
<i>Lama5</i>	-1.5415792
<b>Ctgf</b>	-1.6336439
<i>Tcf15</i>	-1.8120891
<b>Trp63</b>	-1.9570749
<i>Frem2</i>	-2.3166877
<i>Kirrel3</i>	-2.3488613
<b>Hand2</b>	-3.0639499
<i>Pou3f4</i>	-3.2610681
<i>Lin28a</i>	-3.3688949
<i>Alx3</i>	-3.4020552
<i>Foxc1</i>	-3.434487
<i>St14</i>	-4.1221467
<i>Cyp26c1</i>	-4.8790515
<i>Zic5</i>	-5.6682824
<b>Pitx1</b>	-5.9498278
<b>Kynu</b>	-6.1435873
<i>Foxg1</i>	-7.343262
<i>Zic2</i>	-7.3466391
<i>Hmx1</i>	-7.9709667
<b>Zic3</b>	-9.4878579

**Table S9, related to Figure 7.** Gene name, exon coordinates and  $\Delta$ PSI values from *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+/+</sup>* versus *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+Tg</sup>* RNA-seq analysis for skipped exon events with one or more *Srsf3* motifs and a corresponding mouse model with a craniofacial phenotype. Mouse models with a cleft or arched secondary palate are highlighted in bold.

Gene	Exon start	Exon end	$\Delta$ PSI
<i>Ptk2</i>	chr15:73365040	chr15:73365195	41.8
<i>Kars</i>	chr8:112010221	chr8:112010280	38.5
<i>Bcl3</i>	chr7:19809141	chr7:19809259	16.5
<b><i>Tbc1d32</i></b>	chr10:56161134	chr10:56161217	13.7
<b><i>Golgb1</i></b>	chr16:36886130	chr16:36886228	13.1
<b><i>Slc35d1</i></b>	chr4:103208109	chr4:103208212	12.0
<i>Slc4a4</i>	chr5:89232763	chr5:89232860	11.4
<i>B3glct</i>	chr5:149709319	chr5:149709429	10.4
<b><i>Ncor2</i></b>	chr5:125019818	chr5:125020046	10.3
<i>Evc</i>	chr5:37314761	chr5:37314827	10.1
<b><i>Ncoa6</i></b>	chr2:155405474	chr2:155408450	9.5
<i>Ltbp1</i>	chr17:75315002	chr17:75315125	9.0
<i>Ehmt1</i>	chr2:24863771	chr2:24863908	8.8
<i>Gnas</i>	chr2:174328107	chr2:174330434	8.6
<b><i>Ncor2</i></b>	chr5:125124769	chr5:125124980	8.3
<i>Tcirg1</i>	chr19:3897467	chr19:3897681	8.2
<i>Rps6ka3</i>	chrX:159278109	chrX:159278203	7.9
<i>Ltbp1</i>	chr17:75352686	chr17:75352830	7.8
<i>Agap1</i>	chr1:89789162	chr1:89789324	7.6
<b><i>Fuz</i></b>	chr7:44896914	chr7:44896999	7.4
<i>Atpaf2</i>	chr11:60411666	chr11:60411729	7.0
<i>Large1</i>	chr8:72912055	chr8:72912160	6.7
<i>Casp9</i>	chr4:141796437	chr4:141796834	6.0
<i>Casp9</i>	chr4:141796434	chr4:141796834	5.5
<i>Napa</i>	chr7:16112583	chr7:16112630	5.3
<i>Nr2c2</i>	chr6:92152314	chr6:92152417	5.2
<b><i>Trps1</i></b>	chr15:50863882	chr15:50863927	-6.5
<i>Otulin</i>	chr15:27623281	chr15:27623358	-6.8
<i>B3glct</i>	chr5:149725410	chr5:149725487	-7.3
<b><i>Nprl3</i></b>	chr11:32255438	chr11:32255568	-7.7
<i>Mink1</i>	chr11:70609553	chr11:70609717	-7.7
<i>Anapc15</i>	chr7:101897732	chr7:101897817	-8.6
<i>Rhobtb3</i>	chr13:75917692	chr13:75917847	-8.7
<b><i>Tgds</i></b>	chr14:118127494	chr14:118127585	-9.1
<b><i>Dnmt3b</i></b>	chr2:153650784	chr2:153650899	-9.3
<i>Mtf2</i>	chr5:108080824	chr5:108080841	-10.2
<i>Mecom</i>	chr3:29956305	chr3:29956489	-10.2
<i>Uros</i>	chr7:133691085	chr7:133691166	-10.3
<b><i>Nsd2</i></b>	chr5:33821658	chr5:33821728	-10.5

<i>App</i>	chr16:85040250	chr16:85040307	-11.1
<i>Limk2</i>	chr11:3392916	chr11:3393016	-11.5
<i>Clcn5</i>	chrX:7318424	chrX:7318488	-11.5
<i>Ltbp3</i>	chr19:5753928	chr19:5754048	-12.5
<i>Arhgap29</i>	chr3:121973878	chr3:121974115	-12.6
<b><i>Tbx3</i></b>	chr5:119675242	chr5:119675302	-14.7
<i>Clcn5</i>	chrX:7318383	chrX:7318488	-15.7
<b><i>Dzip1l</i></b>	chr9:99632636	chr9:99632748	-16.4
<i>Rps6ka3</i>	chrX:159278109	chrX:159278203	-22.5
<b><i>Zfp950</i></b>	chr19:61118226	chr19:61118347	-23.8
<i>Disp1</i>	chr1:183202951	chr1:183203039	-29.5
<i>Mbtd1</i>	chr11:93891338	chr11:93891388	-38.0

**Table S10, related to Figures 1, 6 and 7.** Primers used in qPCR and qRT-PCR analyses.

<b>Transcript</b>	<b>Forward primer (5' to 3')</b>	<b>Reverse primer (5' to 3')</b>
<b>qPCR primers</b>		
<i>Chrd</i>	GCTATTTTGGATGGTGACCGG	CAGCTCTGATTCTCTGGGAA
<i>Cask</i>	CAGTTTACCCTGCCACCAGT	CCTTCAAGATTGTGCCAAGC
<i>Smad7</i>	CTCCTCCTTACTCCAGATAC	CATTCCCCTGAGGTAGATCA
<i>Srsf3</i>	GAGACCGAGAATCTGTAGGA	CGGGAAAAGCTTCTCCTTCT
<i>Fgfr2</i>	CTCGGGGATAAATAGCTCCA	CTTCTTGGTCGTGGTCTTCA
<i>Fgfr2</i>	CTGCATGGTTGACAGTTCTG	CTTCTTGGTCGTGGTCTTCA
<i>B2m</i>	ACTGACCGGCCTGTATGCTA	TGAAGGACATATCTGACATCTCTA
<i>Melk</i>	GATGTTTGGAGCATGGGCAT	GCGGTGATGTACAGAAAGCT
<i>Limk2</i>	GTGGTCCTTCCTGTGTTGTC	CCCATGGCAGAATTCTCCAA
<i>Dmpk</i>	GTATTAGTGAAAGGGGACCG	CATTCCATCAGGCTGCAGTT
<i>Prkd2</i>	GACTCCTGAGAAGGTATTCG	CAGGTTGTTGATGAGGTCGA
<b>qRT-PCR primers</b>		
<i>Pdgfc</i>	CTGCTCAACAATGCTGTGAC	CCTCTTCCTTGAGGAGATTC
<i>Col2a1</i>	CGCTACACTCAAGTCACTGA	CGGTCTCCATGTTGCAGAAA
<i>Kdr</i>	GTTTGCCTGGCGATTTTCTC	CAGAAGATACTGTCACCACC
<i>Foxd3</i>	CCTCTACCCAATCCTGGACT	CACAGTACTGAGAACACAGG
<i>Zic5</i>	CAAGGCCAAGTACAAGCTCA	GTGGACATGGGAGTGTTTCT
<i>Pou3f4</i>	CAGGACCACTCTGATGAAGA	GAAGCTCAGTTGTAAGGCCT
<i>B2m</i>	ACTGACCGGCCTGTATGCTA	TGAAGGACATATCTGACATCTCTA



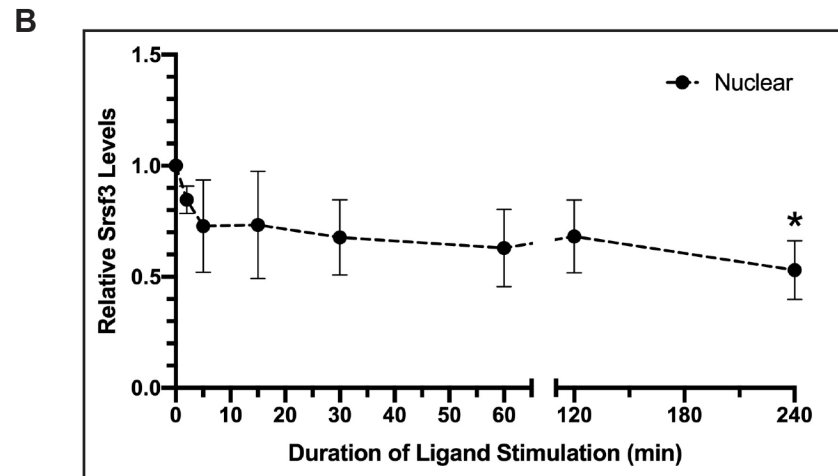
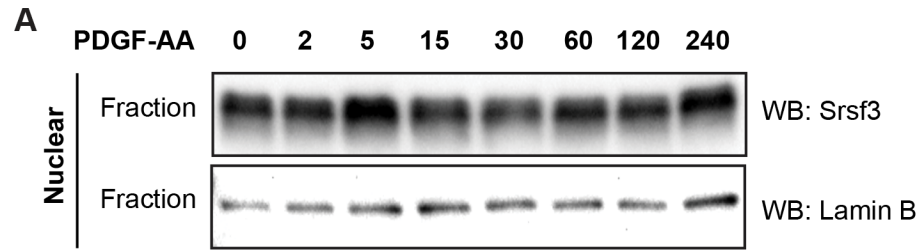
## Supplemental Figure Legends

**Figure S1, related to Figure 3.** Total Srsf3 protein levels in the nucleus do not change in response to PDGF-AA ligand treatment. (A) Western blot analysis of total Srsf3 levels in nuclear fractions of iMEPM cells that were untreated or treated with PDGF-AA ligand in a time course analysis from 2 to 240 minutes. WB, Western blot. (B) Line graph depicting quantification of band intensities from three independent experiments as in (A). Data are presented as mean  $\pm$  SEM. \*,  $p < 0.05$ .

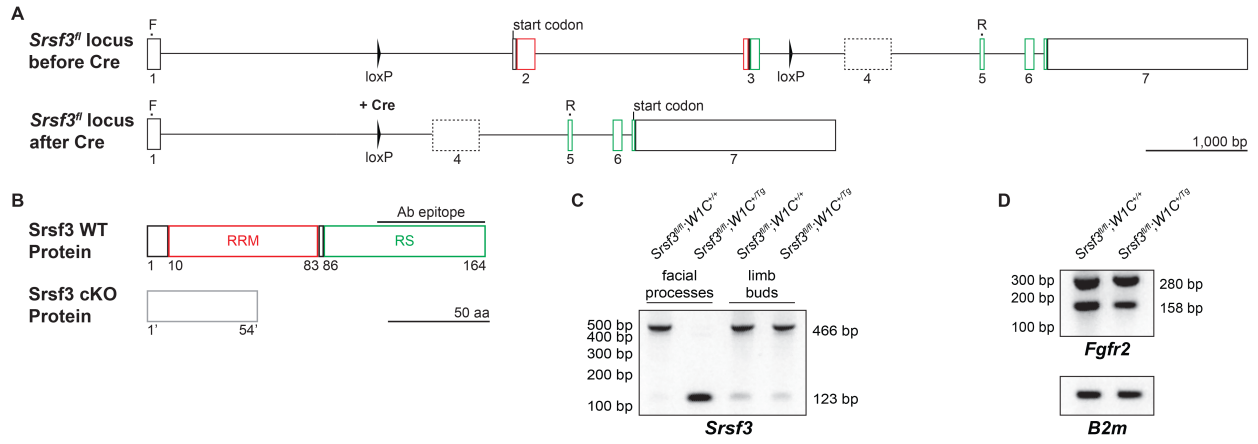
**Figure S2, related to Figure 4.** Efficient deletion of *Srsf3* exons 2 and 3 and maintenance of alternative RNA splicing in *Srsf3* conditional knock-out embryos. (A) Conditional, floxed *Srsf3* locus before (top) and after (bottom) Cre exposure. Boxes represent exons. Colors correspond to protein domains in (B). Black dots indicate sites of forward (F) and reverse (R) primers used in (C). Triangles represent loxP sites. Dotted line surrounds exon 4, which contains a premature termination codon and is excluded from the major transcript. (B) Srsf3 wild-type (top) and conditional knock-out (bottom) proteins. Amino acid residues at the boundaries of the RNA recognition motif (RRM) and arginine/serine-rich (RS) domain are indicated. The epitope for the antibody used in Figure 2, Figure 3 and Figure S1 is indicated. (C) Representative qPCR product gel depicting *Srsf3* transcripts in the facial processes (left) and limb buds (right) of E12.5 *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+/+</sup>* versus *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+Tg</sup>* embryos. (D) Representative qPCR product gel depicting *Fgfr2* and control *B2m* transcripts in the facial processes of E12.5 *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+/+</sup>* versus *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+Tg</sup>* embryos.

**Figure S3, related to Figure 4.** Craniofacial morphological defects in *Srsf3* conditional knock-out embryos. (A-D''') Hematoxylin and eosin-stained coronal sections of E10.5 (A,B) and E12.5 (C-D''') *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+/+</sup>* embryos (top) and *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+Tg</sup>* embryos (bottom). Sections in C-D''' move from anterior to posterior craniofacial structures. LNP, lateral nasal process; MNP, medial nasal process; MxP, maxillary process; MdP, mandibular process, LV, lateral ventricle, NS, nasal septum, TV, third ventricle, PS, secondary palatal shelf, T, tongue. Bars, 1 mm.

**Figure S4, related to Figure 6.** *Srsf3* conditional knock-out RNA-seq reads skip exons 2 and 3. IGV snapshots of *Srsf3* transcripts from RNA-seq analysis of three biological replicates each of E11.5 *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+/+</sup>* (control) versus *Srsf3<sup>fl/fl</sup>;Wnt1-Cre<sup>+Tg</sup>* (cKO) maxillary process mesenchyme.



**Figure S1.**



**Figure S2.**

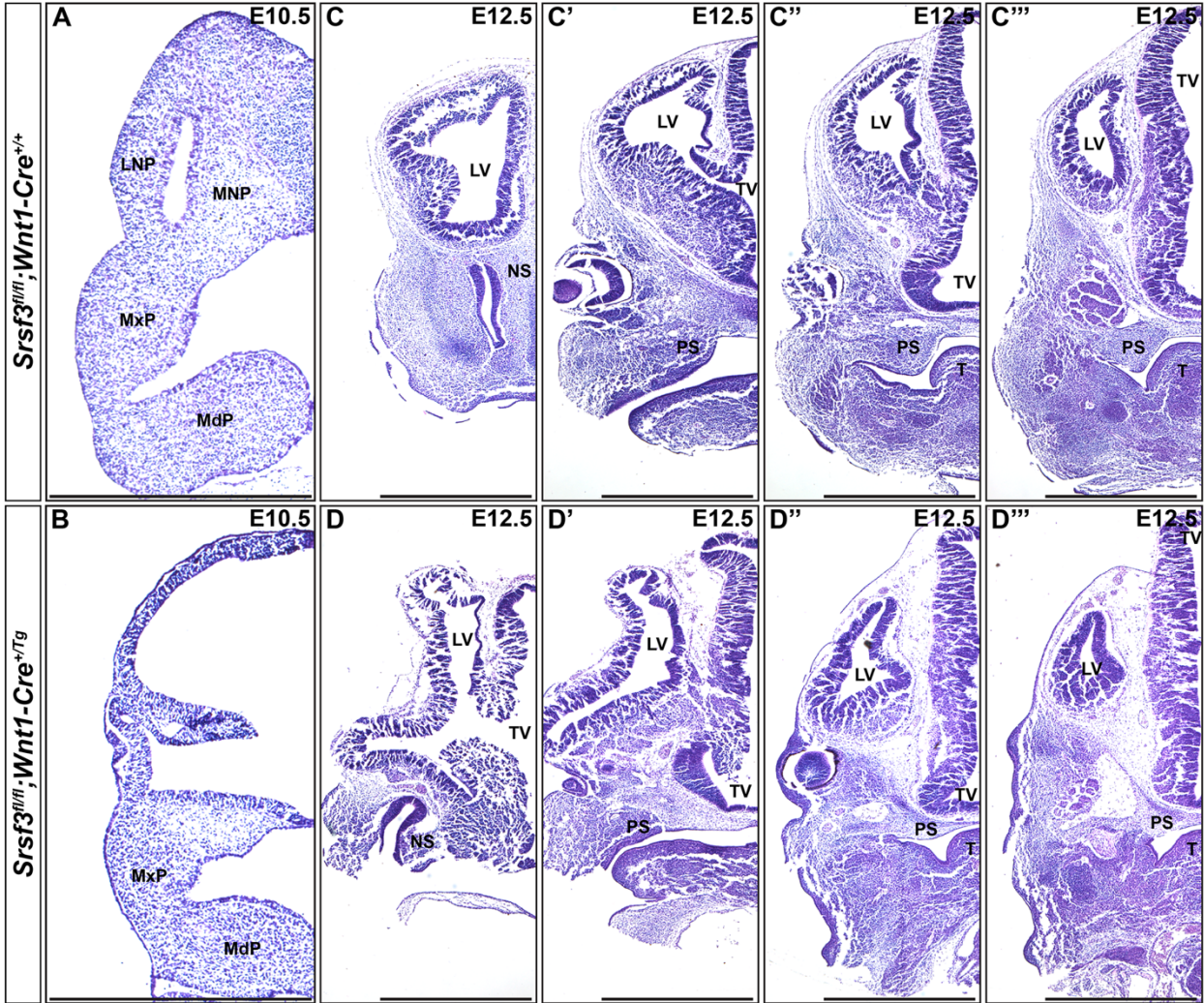
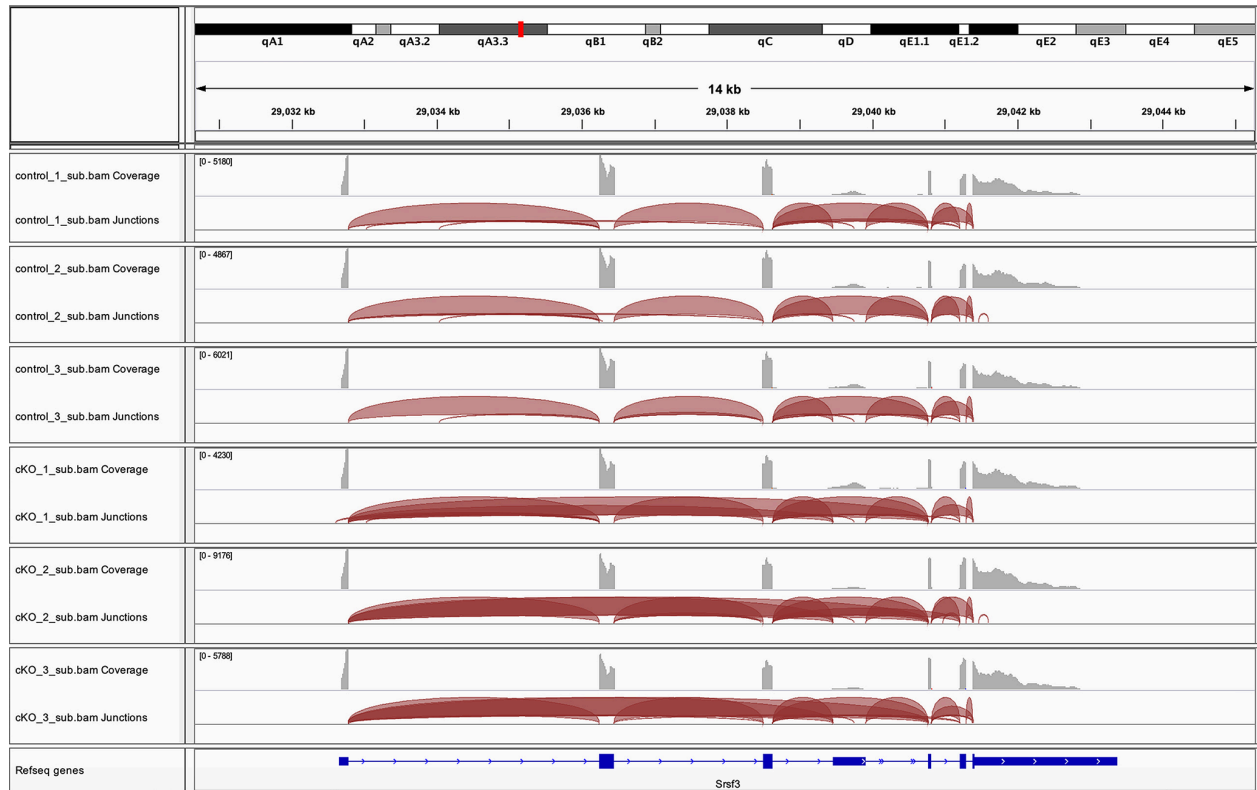


Figure S3.



**Figure S4.**