

Expanded View Figures

Figure EV1. Slc7a5 mRNA expression pattern in E10.5 mouse embryo.

- A E10.5 whole embryo side view and TSs (white dashed lines) showing expression in: (a1) forebrain (fb) and (a1') optic vesicle, including regions of retina (open arrowhead) and lens; (a1, a2) hindbrain (hb), trigeminal ganglion (V) and (a2') otic vesicle and vestibule–cochlear ganglion (VIII) and first branchial arch (ba); (a3, a3') dorsal spinal cord (sc), dorsal root ganglia (drg), dermamyotome (dm), mesonephric duct (mnd).
- B Forelimb bud (fl) (b1) and hindlimb bud (hl) (b2) in mesenchyme below the apical ectoderm ridge (aer) and in the zone of polarising activity (zpa).
- C Forming somite (s) and secondary neural tube (white arrowhead).

Data information: Images representative of n = 4 embryos, scale bars 200 μ m except for sections 100 μ m.

	E9.5	E10.5	
Wildtype	A	B	
Slc7a5-null	c	D	

Figure EV2. A small subset of *Slc7a5*-null embryos exhibits a "flat-top-like" phenotype.

A–D Wild-type littermate (A and B) and *Slc7a5*-null embryos (C and D) were imaged at E9.5 (A and C) or E10.5 (B and D). A subset of *Slc7a5*-null embryos (~ 11%, 4/35 null embryos at E9.5 and 1/6 null embryos at E10.5) shows a "flat-top"-like phenotype with no expansion of the forebrain as described in [27] (see arrowheads). Scale bars 200 μm.



- A-F mRNA in situ hybridisation in E9.5 wild-type and Slc7a5-null embryos for neurogenesis marker genes. FoxG1 and Neurog2 double in situ hybridisation in E9.5 (A-C) wild-type and (D-F) Slc7a5-null embryos (n = 2 each for double FoxG1/Neurog2, a further n = 3 Slc7a5-null andn = 2 wild-type embryos were assessed for *Neurog2* expression in wholemount and sections). Images in (A and D) show frontal view of FoxG1 mRNA detected with fast red (white dotted lines indicate shape of the head). Expression of FoxG1 in rostral-most forebrain was detected in Slc7a5-null embryos with similar intensity but in a reduced domain when compared to wild type. This suggests that Slc7a5 loss does not disrupt brain regionalisation, but leads to a reduction in the amount of tissue and/or failure of morphogenetic events underlying neural tube closure [31]. (B-F) To determine whether neurogenesis was affected in *Slc7a5*-null embryos, we assessed the expression pattern of *Neurogenin-2* (Neurog 2), a neural progenitor marker [32]. Widespread reduction in Neurog2 expression was apparent in whole Slc7a5-null embryos (E, F); some Neurog2-positive cell populations were represented in reduced domains (arrowheads in C, F), while others were absent (asterisk in C, F) (dotted line on the wild-type embryo (C) indicates regions of fore- and midbrain not apparent in the Slc7a5-null embryo).
- G–J Delta1, a marker of newborn neurons [33], was detected in (G, H) wild type and (I, J) Slc7a5-null E9.5 (n = 3 each) in fore- and midbrain but showed a distorted pattern in null embryos due to neurulation failure; TSs through (g1, i1) the forebrain, (g2, i2) at the level of the otic vesicles, (g3, i3) posterior hindbrain and (h1, j1) spinal cord show reduction of the dorsally located Delta1-expressing cells in the Scl7a5-null embryos. Scale bars 200 μm, except sections 100 μm.







					-608
CGCCCTCAGACT		TGGACCAAGT	TGGACTATA	GGGTGCCTC	ACCCCACAAC
-600 -	590	-580	-570	-560	-550
1 1	1	Le	ef1/TCF mot	if	1
CCACCCCCACTT	ATCCTAGAT	AAGATATTTA	TCCTTCATC	CCCAGACGC	ACAGGATATG
-540 -	530	-520	-510	-500	-490
		, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			
	AGCGGAGAI	AAACACCCGC			
-400 -	470	-400	-450	-440	-450
TGGGACTATACA	CTGAGCCAC	CCCCCTTCCG	GTGCTTTCTC	CCTTGACAA	GTCCTCTGCG
-420 -	410	-400	-390	-380	-370
CCGCCTGGGGCG	GGAGAAGAA	GCACGCCCTT	ACCAATCGGC	CCTGACATC	CTGCACAGGA
-360 -	350	-340	-330	-320	-310
		1			
CTTGTGAGGAGG	TGTGAGTCG	ACTCTCCACC	CGCGTACAAC	CATTCGGATC:	TTGTGAAACA
-300 -	290	-280	-270	-260	-250
CGCCGCGATAAA	.CCCATCAGT	AGTCACGCCG	GCGTTAAACAC	CATCAGTAGT	CCTCAGTTTC
-240 -	230	-220	-210	-200	-190
	MAX mot	if			
AGGATCTTAA <mark>CG</mark>	CTCCACGTG	CTCTAA CAGA	CTTGGGGGC	GGGGGCCCGC	GAAGGAGGCG
-180 -	170	-160	-150	-140	-130
GGGTCGCGCGGG	CTGAGGGGG	CGGGGGGCCTT	TGCGAGCTG	ACCAACAGC	AGCGGCGATG
-120 -	110	-100	-90	-80	-70
1 1		Lef1/	TCF motif	MYC motif	
GGCGGAGCCTGG	AGGGCGGGT	AACGG TAGAG	ATAAAGGCC	GCGGGGCG	GG GCGGACGC
-60 -	50	-40	-30	-20	-10
		i	MYC mo	tif	I
AGACGCACTTCT	CAGTACGCG	GAGAGCGGC	CGGCCGCGCG	GCACGCC GGG	FATCCAGGCC
TSS +	10	+20	+30	+40	+50
TSS +		+20	+30	+40	+50
TSS + GAGCCGGGAACG +60 +	10 CCGAGAGCA	+20 TGGCGGTCGC +80	+30 CGGGCGCCAAC +90	+40 GAGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC	+50 GTTGCGACCC +100
TSS + GAGCCGGGAACG +60 +	10 CCCGAGAGCA 70	+20 TGGCGGTCGC +80	+30 CGGGCGCCAAC +90	+40 GAGGCGCGCGCGC +110	+50 GTTGCGACCC +100
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGCGG	10 CCCGAGAGCA 70 CCGGCCGGAGG	+20 TGGCGGTCGC +80 AGGAGCGGCA	+30 CGGGCGCCAAC +90 GGCGCGGGGAC	+40 GAGGCGCGCGC +110 GAAGATGTTGC	+50 GTTGCGACCC +100 GAGGCGCGGC
TSS+GAGCCGGGGAACG+60+CGGCGGCGGCGGCGG+110+	10 Image: CCGAGAGCA 70 Image: CCGGCGGAGG 120 Image: CCGCGAGAGCA	+20 TGGCGGTCGC +80 AGGAGCGGCA +130	+30 CGGGCGCCAAC +90 GGCGCGGGGAC +140	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150	+50 STTGCGACCC +100 SAGGCGCGGC +160
TSS+GAGCCGGGGAACG+60+CGGCGGCGGCGGG+110+GCGGGGACGGCG	10 Image: CCGAGAGCA 70 Image: CGGCGGAGG 120 Image: CGGGACCCCG CCGGGACCCCG Image: CGGACCCCG	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG	+30 CGGGCGCCAAC +90 CGGCGCGGGGAC +140 CGGTGACCCTC	+40 GAGGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAAT2	+50 GTTGCGACCC +100 GAGGCGCGGC +160 ATCACGCTGC
TSS+GAGCCGGGGAACG+60+CGGCGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	10 CCCGAGAGCA 70 CCGGCGGAGG 120 CCGGACCCCG 180 CCGGACCCCG	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190	+30 CGGGCGCCAAC +90 AGGCGCGGGGAC +140 CGTGACCCTC +200	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATZ +210	+50 GTTGCGACCC +100 GAGGCGCGGC +160 ATCACGCTGC +220
TSS+GAGCCGGGGAACG+60+CGGCGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	10 Image: constraint of the second secon	+20 T TGGCGGTCGC +80 A AGGAGCGGCA +130 A AGGGCGAAGG +190 T	+30 CGGGCGCCAAC +90 AGGCGCGGGGAC +140 CGTGACCCTC +200	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATZ +210 CCCCATCTTCC	+50 GTTGCGACCC +100 GAGGCGCGGGC +160 ATCACGCTGC +220
TSS+GAGCCGGGGAACG+60+CGGCGGCGGCGGCGG+110+GCGGGGACGGCG+170+TCAACGGTGTGG+230+	10 Image: Comparison of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGC +190 TGGGCACCAT +250	+30 CGGGCGCCAAC +90 AGGCGCGGGGAC +140 CGTGACCCTC +200 CCATCGGCTCT +260	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATZ +210 CGGCATCTTCC +270	+50 STTGCGACCC +100 SAGGCGCGGGC +160 ATCACGCTGC +220 STGACGCCCA +280
TSS+GAGCCGGGGAACG+60+CGGCGGCGGCGGGGG+110+GCGGGGACGGCG+170+TCAACGGTGTGG+230+	10 Image: constraint of the second secon	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250	+30 CGGGCGCCAAC +90 AGGCGCGGGGAC +140 CGTGACCCTC +200 CATCGGCTCT +260	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATA +210 CGGCATCTTCC +270	+50 STTGCGACCC +100 SAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA +280
TSS+GAGCCGGGAACG+60+CGGCGGCGGCGGG+110+GCGGGGACGGCGG+170+TCAACGGTGTGG+230+CCGGCGTGCTCA	10 Image: Constraint of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG	+30 CGGGCGCCAAC +90 AGGCGCGGGGAC +140 CGTGACCCTC +200 CATCGGCTCT +260 GACTGTCGCTC	+40 SAGGCGCGCGCGC +110 SAAGATGTTGC +150 SCAGCGCAATA +210 CGGCATCTTCC +270 CGTGGTGTGTGGC	+50 STTGCGACCC +100 SAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA +280 GCTGTGTGTGCG
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGG +110 + GCGGGGACGGCGG +170 + TCAACGGTGTGG +230 + CCGGCGTGCTCA +290	10 Image: Constraint of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG +310	+30 CGGGCGCCAAC +90 CGGCGCGGGGAC +140 CGTGACCCTC +200 CATCGGCTCT +260 CATCGGCTCC +320	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATA +210 CGGCATCTTCC +270 CGTGGTGTGGCG +330	+50 STTGCGACCC +100 SAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA +280 GCTGTGTGTGCG +340
TSS+GAGCCGGGAACG+60+CGGCGGCGGCGGGGG+110+GCGGGGACGGCG+170+TCAACGGTGTGG+230+CCGGCGTGCTCA+290+GCGTCTTCTCCA	10 Image: Comparison of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG +310 CACTCTGCTA	+30 GGGCGCCAAC +90 AGGCGCGGGGAC +140 GCGTGACCCTC +200 CATCGGCTCT +260 GACTGTCGCTC +320 ACGCGGAGCTC	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATZ +210 CGGCATCTTCC +270 CGTGGTGTGGC +330 GGGCACCACCZ	+50 STTGCGACCC +100 SAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA +280 SCTGTGTGTGCG +340 ATCTCCAAGT
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGG +110 + GCGGGGACGGCGG +170 + TCAACGGTGTGGG +230 + CCGGCGTGCTCA +290 + GCGTCTTCTCCA +350	10 Image: Constraint of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG +310 CACTCTGCTA +370	+30 :GGGCGCCAAC +90 .GGCGCGGGGAC +140 :CGTGACCCTC +200 :CATCGGCTCT +260 :CATCGGCTCC +320 .CGCGGAGCTCC +380	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATZ +210 CGGCATCTTCC +270 CGTGGTGTGGGC +330 GGGCACCACCZ +390	+50 <u>STTGCGACCC</u> +100 <u>SAGGCGCGGC</u> +160 <u>ATCACGCTGC</u> +220 <u>STGACGCCCA</u> +280 <u>SCTGTGTGTGCG</u> +340 <u>ATCTCCAAGT</u> +400
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGG +110 + GCGGGGACGGCGG +170 + TCAACGGTGTGG +230 + CCGGCGTGCTCA +290 + GCGTCTTCTCCA +350	10 Image: Constraint of the second	+20 T TGGCGGTCGC +80 A AGGAGCGGCA +130 A AGGGCGAAGG +190 T TGGGCACCAT +250 G GGTCGCCCGG +310 A CACTCTGCTA +370 A	+30 CGGGCGCCAAC +90 AGGCGCGGGGAC +140 CGTGACCCTC +200 CATCGGCTCT +260 CATCGGCTCT +260 CATCGGCTCT +320 ACGCGGAGCTC +380	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATZ +210 CGGCATCTTCC +270 CGTGGTGTGGC +330 GGGCACCACCZ +390	+50 STTGCGACCC +100 GAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA F280 GCTGTGTGTGCG +340 ATCTCCAAGT +400
TSS+GAGCCGGGGAACG+60+CGGCGGCGGCGGCGG+110+GCGGGGACGGCGG+170+TCAACGGTGTGG+230+CCGGCGTGCTCA+290+GCGTCTTCTCCA+350+CAGGCGGCGACT+410	10 Image: Constraint of the second	+20 T TGGCGGTCGC +80 A AGGAGCGGCA +130 A AGGGCGAAGG +190 T TGGGCACCAT +250 G GGTCGCCCGG +310 C CACTCTGCTA +370 T TGCTGGAGGT	+30 CGGGCGCCAAC +90 AGGCGCGGGGAC +140 CGTGACCCTC +200 CATCGGCTCT +260 CATCGGCTCT +320 ACGCGGAGCTC +380 CCTACGGCTCC	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATZ +210 CGGCATCTTCC +270 CGTGGTGTGGGC +330 GGGCACCACCZ +390 GTTGCCCGCCC	+50 STTGCGACCC +100 GAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA +280 GCTGTGTGTGCG +340 ATCTCCCAAGT +400 FTCCTCAAGC
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TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	10 Image: Constraint of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG +310 CACTCTGCTA +370 TGCTGGAGGT +430 TTCGGCCCTC	+30 CGGGCGCCAAG +90 AGGCGCGGGGAG +140 CGTGACCCTC +200 CATCGGCTCT +260 CATCGGCTCT +320 ACGCGGAGCTC +380 CTACGGCTCC +440 CCTCGCAGTAC	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATZ +210 CGGCATCTTCC +270 CGTGGTGTGGGC +330 GGGCACCACCZ +390 GTTGCCCGCCT +450 CATCGTGGCGC	+50 STTGCGACCC +100 SAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA +280 SCTGTGTGTGCG +340 ATCTCCAAGT +400 STCCTCAAGC +460 CTGGTCTTCG
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	10 Image: Comparison of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG +310 CACTCTGCTA +370 TGCTGGAGGT +430 TTCGGCCCTC +490	+30 CGGGCGCCAAG +90 CGCGCGCGGGAG +140 CCTCGGCTCT +200 CCATCGGCTCT +260 CCATCGGCTCT +320 CCACGGGAGCTC +320 CCCCGCAGTAC +440 CCTCGCAGTAC +500	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATZ +210 CGGCATCTTCC +270 CGTGGTGTGGGGC +330 GGGCACCACCZ +390 GTTGCCCGCCC +450 CATCGTGGCGC +510	+50 GTTGCGACCC +100 GAGGCGCGGC +160 ATCACGCTGC +220 GTGACGCCCA +280 GCTGTGTGTGCG +340 ATCTCCAAGT +400 FTCCTCAAGC +460 CTGGTCTTCG +520
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	10 Image: Constraint of the second	+20 T TGGCGGTCGC +80 A AGGAGCGGCA +130 A AGGGCGAAGG +190 T TGGGCACCAT +250 G GGTCGCCCGG +310 C CACTCTGCTA +370 T TGCTGGAGGT +430 T TCGGCCCTC +490 T TCTTCCCCAC	+30 CGGGCGCCAAG +90 CGCGCGCGGGAG +140 CCTCGGCTCT +200 CATCGGCTCT +260 CATCGGCTCT +320 CCATCGGCTCC +320 CCCCGCGAGCTCC +440 CCTCGCAGTAC +500	+40 <u>GAGGCGCGCGCGC</u> +110 <u>GAAGATGTTGC</u> +150 <u>GCAGCGCAATZ</u> +210 <u>CGGCATCTTCC</u> +270 <u>CGTGGTGTGGGGC</u> +330 <u>GGGCACCACCZ</u> +390 <u>GGGCACCACCZ</u> +450 <u>CAGCGCGCGC</u> +510 <u>GCCCGACGACC</u>	+50 GTTGCGACCC +100 GAGGCGCGGC +160 ATCACGCTGC +220 GTGACGCCCA +280 GCTGTGTGTGCG +340 ATCTCCAAGT +400 FTCCTCAAGC +460 CTGGTCTTCG +520 GCTGCCCAAGC
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	10 Image: Constraint of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG +310 CACTCTGCTA +310 TGCTGGAGGT +430 TTCGGCCCTC +490 TCTTCCCCAC +550	+30 CGGGCGCCAAC +90 CGCGCGCGGGAC +140 CCGTGACCCTC +200 CATCGGCTCT +260 CATCGGCTCT +320 CCATCGGCAGTAC +320 CCTACGGCAGTAC +440 CCTCGCAGTAC +500 CTTGTCCGGTC +560	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATA +210 CGGCATCTTCC +270 CGTGGTGTGGGC +330 GGGCACCACCA +390 GGGCACCACCA +390 GTTGCCCGCCC +450 CATCGTGGCGC +510 GCCCGAGGAAC	+50 GTTGCGACCC +100 GAGGCGCGGGC +160 ATCACGCTGC +220 GTGACGCCCA +280 GCTGTGTGTGCG +340 ATCTCCAAGT +400 FTCCTCAAGC +460 CTGGTCTTCG +520 GCTGCCAAGC +580
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	10 Image: Constraint of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG +310 CACTCTGCTA +310 TGCTGGAGGT +430 TTCGGCCCTC +490 TCTTCCCCAC +550	+30 CGGGCGCCAAC +90 CGCGCGCGGGAC +140 CCTCGGCTCT +200 CATCGGCTCT +260 CATCGGCTCT +320 CCTCGCGGAGCTC +320 CCTACGGCTCC +440 CCTCGCAGTAC +500 CTTGTCCGGTC +560	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATA +210 CGGCATCTTCC +270 CGTGGTGTGGGC +330 GGGCACCACCA +390 GGGCACCACCA +450 CATCGTGGCGC +510 GCCCGAGGAAC +570	+50 GTTGCGACCC +100 GAGGCGCGGGC +160 ATCACGCTGC +220 GTGACGCCCA +280 GCTGTGTGTGCG +340 ATCTCCAAGT +400 FTCCTCAAGC +460 CTGGTCTTCG +520 GCTGCCAAGC +580
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGG +110 + GCGGGGACGGCG +170 + TCAACGGTGTGG +230 + CCGGCGTGCTCA +290 + GCGTCTTCTCCA +350 + CAGGCGGCGACT +410 + TCTGGATCGAGC +470 + CCACCTACTTGC +530 +	10 Image: Constraint of the second	+20 T TGGCGGTCGC +80 A AGGAGCGGCA +130 A AGGGCGAAGG +190 T TGGGCACCAT +250 G GGTCGCCCGG +310 C CACTCTGCTA +370 G TGCTGGAGGT +430 T TCCGGCCCTC +490 T TCTTCCCCAC +550 G GTAAGTAGGG	+30 CGGGCGCCAAC +90 CGCGCGCGGGAC +140 CCGTGACCCTC +200 CATCGGCTCT +260 CATCGGCTCT +260 CCATCGGCTCC +320 CCGCGGAGCTC +320 CCTCCGCAGTAC +440 CCTCGCAGTAC +500 CTTGTCCGGTC +560 CCTCCGGGCTC	+40 GAGGCGCGCGCGC +110 GAAGATGTTGC +150 GCAGCGCAATA +210 CGGCATCTTCC +270 CGTGGTGTGGGGC +330 GGGCACCACCA +390 GGGCACCACCA +450 CATCGTGGCGCGC +510 GCCCGAGGAAC +570 GGGGTGGGGGCC	+50 STTGCGACCC +100 SAGGCGCGGGC +160 ATCACGCTGC +220 STGACGCCCA +280 STGACGCCCA +340 ATCTCCAAGT +400 STCCTCAAGC +460 CTGGTCTTCG +520 SCTGCCAAGC +580 CGGGCTGGGGG
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGG +110 + GCGGGGACGGCGG +170 + TCAACGGTGTGGG +230 + CCGGCGTGCTCA +290 + GCGTCTTCTCCA +350 + CAGGCGGCGACT +410 + TCTGGATCGAGC +470 + CCACCTACTTGC +530 + TCGTGGCCTGCCC +590 +	10 Image: Constraint of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG +310 CACTCTGCTA +370 TGCTGGAGGT +430 TTCGGCCCTC +490 TCTTCCCCAC +550 GTAAGTAGGG +610	+30 CGGGCGCCAAC +90 CGCGCGCGGGAC +140 CCTCGGCTCT +200 CATCGGCTCT +260 CATCGGCTCT +320 CGCGGGAGCTC +320 CCTCGCGGAGCTC +440 CCTCGCAGTAC +500 CTTGTCCGGTC +560 CGTCCGGGCTC +620	+40 <u>GAGGCGCGCGCGC</u> +110 <u>GAAGATGTTGC</u> +150 <u>GCAGCGCAAT</u> +210 <u>CGGCATCTTCC</u> +270 <u>CGTGGTGTGGGC</u> +330 <u>GGGCACCACC</u> +330 <u>GGGCACCACC</u> +390 <u>GGGCACCACC</u> +450 <u>CATCGTGGCGC</u> +570 <u>GGGGTGGGGGCC</u> +630	+50 STTGCGACCC +100 SAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA +280 STGACGCCCA +340 ATCTCCAAGT +400 STCCTCAAGC +460 CTGGTCTTCG +580 CGGGCTGGGG +640
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	10 Image: Constraint of the second	+20 T TGGCGGTCGC +80 A AGGAGCGGCA +130 A AGGGCGAAGG +190 T TGGGCACCAT +250 G GTCGCCCGG +310 C CACTCTGCTA +370 G TGCTGGAGGT +430 T TCCGGCCCTC +490 A TCTTCCCCAC +550 G GTAAGTAGGG +610 C CAAGGTTCCT	+30 CGGGCGCCAAC +90 CGCGCGCGGGAC +140 CCTCGGCTCT +200 CATCGGCTCT +260 CATCGGCTCT +260 CATCGCCGCAGTAC +320 CCTCGCAGTAC +30 CCTCGCAGTAC +440 CTTGTCCGGTC +500 CTTGTCCGGTC +500 CTTGTCCGGTC +500 CCTCGCAGTAC CCCCGCGCCCC +500 CCTCGCAGTAC CCCCGCGCCCCC CCCCGCGCCCCCC CCCCCGCCCCCCCC	+40 <u>GAGGCGCGCGCGC</u> +110 <u>GAAGATGTTGC</u> +150 <u>GCAGCGCAAT</u> +210 <u>CGGCATCTTCC</u> +270 <u>CGTGGTGTGGGC</u> +330 <u>GGCACCACC</u> +330 <u>GGCACCACC</u> +30 <u>GGCACCACC</u> +450 <u>CATCGTGGCGC</u> +570 <u>GGGTGGGGGCC</u> +630 <u>GCTCGGCAAC</u>	+50 STTGCGACCC +100 SAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA +280 STGACGCCCA +340 ATCTCCAAGT +400 STCCTCAAGC +460 CTGGTCTTCG +580 CGGGCTGGGG +640 ACCCATCCCA
TSS + GAGCCGGGAACG +60 + CGGCGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	10 Image: Constraint of the second	+20 TGGCGGTCGC +80 AGGAGCGGCA +130 AGGGCGAAGG +190 TGGGCACCAT +250 GGTCGCCCGG +310 CACTCTGCTA +370 TCGGGCCCTC +490 TCTTCCCCAC +550 GTAAGTAGGG +610 CAAGGTTCCT +670	+30 CGGGCGCCAAC +90 CGCGCGCGGGAC +140 CCTCGGCTCT +200 CATCGGCTCT +260 CATCGGCTCT +260 CATCGCCGCAGTAC +320 CCTACGGCTCC +320 CCTACGGCTCC +440 CCTCGCAGTAC +500 CTTGTCCGGTCC +560 CGCGTTTACAC +680	+40 <u>SAGGCGCGCGCGC</u> +110 <u>SAAGATGTTGC</u> +150 <u>SCAGCGCAAT</u> +210 <u>CGGCATCTTCC</u> +270 <u>CGTGGTGTGGGC</u> +330 <u>SGGCACCACC</u> +330 <u>SGGCACCACC</u> +390 <u>STTGCCCGCC</u> +450 <u>SCCCGAGGAAC</u> +570 <u>SGGGTGGGGGCC</u> +630 <u>SCTCGGCAAC</u> +690	+50 STTGCGACCC +100 SAGGCGCGGC +160 ATCACGCTGC +220 STGACGCCCA +280 STGACGCCCA +340 ATCTCCAAGT +400 STCCTCAAGC +460 CTGGTCTTCG +580 CGGGCTGGGG +580 CGGGCTGGGG +640 ACCCATCCCA +700

Figure EV4. Position and sequence of MYC and LEF/TCF binding sites in *Slc7a5* promoter region.

GATCATTT

Analysis of the -1,075 to +352 bp sequence of the Slc7a5 mouse gene with the MatInspector program [61] identified putative MYC binding sites at positions TSS -9/-25 and +30/+46 (with a matrix similarity comprised between 0.925 and 0.931). A further MYC/ MAX binding site, similar to the MYC binding E-box motif identified in human Slc7a5 promoter [19], was found at -TSS -154/-170. A potential LEF/TCF binding motif (sequence tagagataAAAGgccgc) at position -18/-34 from the transcription starting site. It showed a matrix similarity of 0.903 (see Dataset EV3). This motif was also found by analysing the -1,000 to +100 bp sequence of murine Slc7a5, using the eukaryotic promoter database (http://epd.vitalit.ch/) which is based on the JASPAR CORE 2018 vertebrate database [108]. Another motif was found at position -505 with the cut-off value set at $P \leq$ 0.001. Both sites were highlighted on the murine Slc7a5 gene sequence based on position and matrix similarity.

	Slc7a5						
Littermate	A Contraction of the second se	a1 a3 a2	a2	a3			
Sp5/Sp8 dKO	B b2 b3	b1	b2 A	b3			

Figure EV5. Expression of *Slc7a5* in Sp5/Sp8 double-mutant embryos.

A, B Slc7a5 is expressed to the caudal tip of the Sp5/Sp8 double knockout embryos despite failure to form paraxial mesoderm. (A, a1) Slc7a5 expression in littermate control, (a2, a3) in TS, and (B, b1) in Sp5/Sp8 double knockout (dKO) embryo, (b2, b3) in TS (n = 5/5 mutant embryos and n = 3/3 littermate controls). Open arrowheads indicate posterior neural tube. Scale bars 200 µm, except sections 50 µm.