

Supplementary Material

Analysis of Expression Pattern and Genetic Deletion of *Netrin5* in the Developing Mouse.

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Supplementary Figures 🛛

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Netrin5 mouse Netrin5 human Netrin1 mouse Netrin3 mouse Netrin4 mouse	<u>M T</u> [D Y M M P 	R T R A T W 	L F V W L W	10 S S E A G I 	5 P L L - M	G A A A L T G S	G S L A A G C A	T A T R	V T V A L S L L	20 T P M P C L A A L L	$\begin{bmatrix} I & T \\ V & T \\ V & G \\ L & S \\ W & G \end{bmatrix}$		S - A - V R S -	G	 	30 G L 		L L L L M F 	L L A P A	LS LG GQ GL VA	Q Q A P A (A T A T A Q A S G L	$\begin{bmatrix} 40\\ S\\ A\\ P\\ \end{bmatrix} \\ \begin{bmatrix} A\\ N\\ \end{bmatrix} $
Netrin5 mouse Netrin5 human Netrin1 mouse Netrin3 mouse Netrin4 mouse	D P D P D P D P G V	C Y C Y C S C Y A G	D P D P D E D E A N	G Q Q G A G A R S R	50 R F R F H F C E	R Q R R R R R R R R	FC FC RC SC AC	L P L P I P I P N P	$ \begin{array}{c} P \\ P \\ D \\ G \\ R \\ \end{array} $	V T V T F V L V M G	60 Q L Q L N A N A N L	V G A A A F A L A L	K V G G G G	A A A A K D R E R K	A S V I V I L	PC C RV LA RA	70 S Q P Q S S S S D 1	P T D A T T M	C A C A C G C G C G	L L R R Q	PA SP PP SA NA	A I G I A I N I T I	S - N H R Y R - E L	80 - - C - F
Netrin5 mouse Netrin5 human Netrin1 mouse Netrin3 mouse Netrin4 mouse	 V V C F	 S E Y S	 R G E N	 E E A D	90 - R - - - L - - - - - - - - - - - - - -	2 	 S C R Q	 H L - V P K		N S D S D K	100 S D S D C N	P R P Q A A	K R H	 A H A H S H	P I S Z L Z	 P A A D A H	110 F L L L P P		 D L S A A M	- N P A	 N P G T D S	- H 1 A 1 S 1	 N L S P F R	120 - - T L F
Netrin5 mouse Netrin5 human Netrin1 mouse Netrin3 mouse Netrin4 mouse	C W C W P R	QS RS TW	- L E N D L W Q	- P G A Y L L Q S A	130 G H R E Q H Q A E L) P A F P . F P . A P . D V .	CN CN HN FN HR	SS GS VT VT EK	L L L L I	TL TL TL TV QL	140 D L A L S L P L D L	DG GG K GK EA	S P . C K . C A . E .	F L F L F E F E F Y	L 7 L 7 V 7 L V F 1	TS TS TY VF TH	150 V T V S V S V S L I	$\begin{array}{c} & \\ & \\ & \\ & \\ & \\ & \\ & \\ & \\ & \\ & $	R F R F Q F R F V F	C C C K	TA TP SP SA SP	G 1 G 1 R 1 P 1 R 1	PPP PPE PE PA	160 A A S S A
Netrin5 mouse Netrin5 human Netrin1 mouse Netrin3 mouse Netrin4 mouse	L V L I M A V A M V	L S L S I Y L L L D	A A A A K S K S R S	W A W A M D Q D Q D	170 T S V H C F C	G G G G R G R G K	PW PW TW SW TW	R P R L V P V P K P		WR WH QF GF KY	180 <i>R P</i> <i>R P</i> <i>Y S</i> <i>F S</i> <i>F A</i>	T S S T N		 R K T L S A	M]] D]] T]	YN YG FG	190 R - R L 	P P P	 H R A P 	A A	 P I D G - L	T 1 P 1 E 1	2 K Q S G D D	200 - - N P V
Netrin5 mouse Netrin5 human Netrin1 mouse Netrin3 mouse Netrin4 mouse	E Q G P V K	EA EA KG	V C L C A I	 T D F P C T	210) I T . -	D M Q A S N	R P Q P P F		S G G C T	220 G L G L G G	- A - A I A L A E V	W W F F V I	PG PG ST SV FR	A 1 A 1 L 1 Q 1 A 1	L G L G D G D G L S	230 G P G P R P S P P P	K E S Q Y	K V R V A H G L D I	T T D D E	FH FH FD LD NP	S 1 S 1 N 1 Y 1	P P F P S P S P S A	240 G G V V K
Netrin5 mouse Netrin5 human Netrin1 mouse Netrin3 mouse Netrin4 mouse	P K P K L Q L Q V Q	T R A T D W D W E Q	I V V A V T V T L K	A S A S A T A T I T	250 Y 1 H 1 D 1 N 1	2 R 2 R 7 R 7 R 7 R	V E V E V A I V V R	FG FG FS LT LL	G G R R R K	KA QA LH PA RQ	260 G L G L T F I Q S C	V 1 A A G D G D P C	$\begin{bmatrix} T \\ A \\ E \\ T \\ C \\ Q \end{bmatrix}$	G - G - N E R D I N	D 1 G (D 1	 D S G V L N	270 E L T V A K	A P P	 R D H H		Y Y Y S M H	Y Y Y Y	2 - - - - - - - - - - - - -	280 - - - - - - - - - - - - - - - - - - -

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Netrin5 mouse	-	-	-	V	R	G	R	C	ϱ	С	H	G	H	A	A	R	С	A	T	R	A	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	R	C	R
Netrin5 human	-	-	-	L	R	G	R	C	ϱ	С	H	G	H	A	A	R	С	A	A	R	A	R	-	-	-	-	-	-	-	-	-	-	-	-	-	P	P	R	C	H
Netrin1 mouse	D	L	Q	V	G	G	R	C	K	C	N	G	H	A	A	R	С	V	R	D	R	D	D	-	-	-	-	-	-	-	-	-	-	-	-	S	L	V	C	D
Netrin3 mouse	E	L	Q	V	G	G	R	C	K	С	N	G	H	A	S	R	C	L	L	D	T	\boldsymbol{H}	G	-	-	-	-	-	-	-	-	-	-	-	-	H	L	V	C	D
Netrin4 mouse	D	F	Ι	V	K	G	S	С	F	С	N	G	H	A	D	Q	C	L	P	V	E	G	F	R	P	Ι	K	A	P	G	A	F	H	V	V	H	G	R	C	М

								3	30									3	40									3	50									36	30
Netrin5 mouse	C R	H	H	T	T	G	P	G	C	E	S	C	R	P	<i>s</i>	H	R	D	W	P	W	R	P	A	T	P	Q	H	-	-	P	H	P	C	L	P	С	S	C
Netrin5 human	C R	H	H	T	T	G	P	G	C	E	<i>S</i>	C	R	P	S	H	R	D	W	P	W	R	P	A	T	P	R	Η	-	-	P	H	P	C	L	P	C	S	С
Netrin1 mouse	CR	H	N	T	A	G	P	E	C	D	R	C	K	P	F	H	Y	D	R	P	W	Q	R	A	T	A	R	E	-	-	A	N	E	C	V	A	C	N	С
Netrin3 mouse	C Q	H	G	T	E	G	P	D	С	S	R	С	K	P	F	Y	C	D	R	P	W	Q	R	A	T	G	Q	E	-	-	A	H	A	<i>C</i> .	L	A	C	S	С
Netrin4 mouse	C K	H	N	T	A	G	S	H	C	\mathcal{Q}	H	С	\boldsymbol{A}	P	L	Y	N	D	R	P	W	E	A	A	D	G	R	T	G	A	P	N	E	C	R	T	C	K	С

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Netrin5 mouse	N	ϱ	H	A	R	R	С	R	F	N	S	E	L	F	R	L	S	G	G	R	S	G	G	V	C	E	R	C	R	H	\boldsymbol{H}	T	A	G	R	H	С	H	Y	С
Netrin5 human	N	Q	H	A	R	R	С	R	F	N	S	E	L	F	R	L	S	G	G	R	S	G	G	V	C	E	R	С	R	H	Η	T	A	G	R	H	С	H	Y	С
Netrin1 mouse	N	L	H	A	R	R	С	R	F	N	М	E	L	Y	K	L	S	G	R	K	S	G	G	V	C	L	N	С	R	H	N	T	A	G	R	H	С	H	Y	С
Netrin3 mouse	N	G	H	A	R	R	C	R	F	N	М	E	L	Y	R	L	S	G	R	R	S	G	G	V	C	\boldsymbol{L}	N	C	R	H	N	T	A	G	R	H	C	H	Y	С
Netrin4 mouse	N	G	H	A	D	T	С	H	F	D	V	N	\boldsymbol{V}	W	E	A	S	G	N	R	S	G	G	V	C	N	N	С	Q	H	N	T	E	G	ϱ	H	C	Q	R	С

									4	10									4	20									4	30									4	40
Netrin5 mouse	Q	P	G	F	W	R	D	P	S	<i>Q</i>	P	Ι	T	S	H	K	A	С	R	A	C	ϱ	C	H	P	Ι	G	-	-	-	-	-	-	A	T	G	G	M	С	N
Netrin5 human	ϱ	P	G	F	W	R	D	P	S	Q	P	Ι	F	S	R	R	A	С	R	A	C	Q	C	H	P	Ι	G	-	-	-	-	-	-	A	T	G	G	T	С	N
Netrin1 mouse	K	E	G	F	Y	R	D	М	G	K	P	Ι	T	H	R	K	A	С	K	A	C	D	C	H	P	V	G	-	-	-	-	-	-	A	\boldsymbol{A}	G	K	T	С	N
Netrin3 mouse	R	E	G	F	Y	R	D	P	G	R	V		S	D	R	R	A	С	R	A	C	D	C	H	P	V	G	-	-	-	-	-	-	A	A	G	K	T	C	N
Netrin4 mouse	K	P	G	F	Y	R	D	L	R	R	P	F	S	A	P	D	A	С	K	A	С	S	C	H	P	V	G	S	A	Ι	L	P	F	S	S	V	T	F	C	D

									4	50									4	60									4	70									4	80
Netrin5 mouse	Q	T	S	G	Q	C	S	С	K	L	G	V	T	G	L	T	С	N	R	С	G	P	G	Y	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Netrin5 human	Q	T	S	G	Q	C	T	С	K	L	G	V	T	G	L	T	С	N	R	С	G	P	G	Y	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Netrin1 mouse	2	T	T	G	Q	C	P	С	K	D	G	V	T	G	Ι	T	С	N	R	С	A	K	G	Y	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Netrin3 mouse	Q	T	T	G	Q	C	P	С	K	D	G	V	T	G	L	T	C	N	R	C	A	P	G	F	Q	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Netrin4 mouse	P	S	N	G	D	C	P	С	K	P	G	V	A	G	Р	H	C	D	R	C	М	V	G	Y	W	G	F	G	D	Y	G	С	R	P	С	D	C	A	G	S

									4	90									£	500									5	10									5	20
Netrin5 mouse	-	-	-	-	-	-	-	-	-	2	S	R	S	P	R	М	P	С	$]\varrho$	R	Ι	P	E	A	T	T	T	P	A	T	T	-	P	V	A	S	R	S	D	P
Netrin5 human	-	-	-	-	-	-	-	-	-	Q	S	R	S	P	R	M	P	С	Q	R	Ι	P	E	A	T	T	T	L	A	T	T	-	P	G	A	Y	S	S	D	Р
Netrin1 mouse	-	-	-	-	-	-	-	-	-	Q	S	R	S	P	Ι	A	P	С	1	K	Ι	P	V	A	P	Р	T	T	A	A	S	-	S	V	E	E	-	-	P	E
Netrin3 mouse	-	-	-	-	-	-	-	-	-	Q	S	R	S	P	V	A	P	С	V	K	T	P	V	P	G	P	T	E	E	S	S	-	P	V	Ε	-	-	-	P	Q
Netrin4 mouse	С	D	P	L	T	G	D	С	Ι	S	S	N	A	D	V	D	W	Y	H	E	V	P	A	F	H	S	М	H	N	K	S	E	P	S	W	E	W	E	D	E

								5	30									5	540									5	50									50	60
Netrin5 mouse	Q C	ϱ	G	Y	C	N	V	S	-	-	-	-	-	V	S	S	V	H	М	S	L	ϱ	R	Y	C	Q	ϱ	D	Y	V	L	H	A	Q	V	S	A	S	S
Netrin5 human	QC	\mathcal{Q}	N	Y	C	N	М	S	-	-	-	-	-	D	T	R	V	H	M	S	L	R	R	Y	C	Q	Q	D	Η		L	R	A	Q	V	L	A	S	-
Netrin1 mouse	D	D	S	Y	C	K	A	S	-	-	-	-	-	K	G	K	L	K	M	N	М	K	K	Y	C	R	K	D	Y	A	V	Q	Ι	H	Ι	-	L	K	A
Netrin3 mouse	D C	E	S	H	<u>C</u>	R	P	A	-	-	-	-	-	R	G	S	Y	R	I	S	L	K	K	F	C	R	K	D	Y	A	V	Q	V	A	V	G	A	R	\boldsymbol{G}
Netrin4 mouse	Q G	F	S	A	L	R	H	S	G	K	C	E	C	K	E	Q	V	L	G	N	P	K	A	F	C	G	M	K	Y	S	Y	V	L	K	Ι	K	Ι	L	S

		570		580	590		600
Netrin5 mouse	SOPSEA	VGPEW W	R L A V H V	LAVFKO		RGGOEA	WVP R
Netrin5 human	E A	AAGPAWQ	RLAVRV	LAVYKQ	RAQPVF	RGDQDA	WVP R
Netrin1 mouse	D H	A G - DWW	KFTVNI	ISVYKQ	GTSRIF	RGDQSI	WIRS
Netrin3 mouse	E A	RG - SWT	R F P V A V	LAVFRS	GEERAF	RGSSAL	WVPT
Netrin4 mouse	A I	H D K G S H A	EVNVKI	KKVLKS	TKLKII	RGKRTI	YPES
N					630		640
Netrin5 mouse		GCLRLR	P G A D Y L P C T D Y L		QIHDDL	DNY-DPA	KLIL
Netrin1 mouse			PGIDIL				GLVA
Netrin3 mouse		CCPRL	PGRRVL		G AAAG	TAGRGC	GLSA
Netrin4 mouse	WTNRG	CTCPILN	PGLEYL	VAGHED	VRTG -		KLIV
		650		660	670		680
Netrin5 mouse	N R H G L A	A L P W R P R	WARPLR	RLQQKE	RGGACF	GLLPPT	R S P G
Netrin5 human	DRHGLA	A L P W R P R	WARPLK	RLQQEE	RAGGCF	GVRAP1	P SP R
Netrin 1 mouse			WARKLK	K F Q Q R E			
Netrin ⁴ mouse			W I K K L K	K L Q K K E		r I 🗛	
Netrin4 mouse							
Netrin5 mouse	P R N						
Netrin5 human	PEH						
Netrin1 mouse	The state						
Netrin3 mouse							
Netrin4 mouse							
В							
45.684Mb	4	5.686Mb	45.688Mb	45.690Mb	45.	592Mb	45.694Mb
			B3				
Ntn5-00	l >	_		•	$\nabla^{}$		
	p5-002 >					-	
pro	otein coding						
Nt	n5-003 >					-	
pn	otein coding		10107010				

Figure S1: Netrin5 is a classic Netrin. Protein sequence alignment shows a high level of similarity between Netrin5 and other classic Netrins. Similar or identical residues are boxed. Sequence corresponding to the exon omitted in the short transcript is highlighted in blue (A). The gene structure of mouse *Ntn5* from the Ensembl genome browser (**B**). The *Ntn5*-002 gene structure was experimentally validated. A second alternatively spliced isoform that also includes exon 3 of the *Ntn5*-001 gene model introduced into the *Ntn5*-002 transcript was also found. Other transcripts corresponding to gene prediction models may exist at low levels, but they were not found experimentally or in EST databases. NCBI gene and transcript models XM_006540887.2, XP_006540950.1 and XM_006540890.2, XP_006540953.1 do correctly predict the long and short Ntn5 transcripts we identified, respectively.

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Figure S2: Other *Ntn5* expression. Netrin5 is expressed adjacent to the brain at E11.5 (A,B) and E17.5 (D). There was also expression associated with the olfactory system (B,D arrow), at the tail (A,C) and at the base of whiskers in the chin (D,E). Scale bar is 1.5 mm in A,D and 400 mm in B,C,E.

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Figure S3: *Ntn5* expression abolished without disrupting *Sec1* expression in Ntn5^{-/-} embryos. QPCR analysis of Ntn5 and Sec1 transcripts from E13.5 $Ntn5^{+/+}$, $Ntn5^{+/-}$, and $Ntn5^{-/-}$ whole embryos demonstrates that Ntn5 transcripts were effectively removed in null mutants (A,C), while Sec1 transcripts were not significantly reduced (B,D). Dissociation curves of the Ntn5 products demonstrated that late-arising PCR products from $Ntn5^{-/-}$ samples were different from the normal product in $Ntn5^{+/+}$ and $Ntn5^{+/-}$ samples. A and B show individual reactions from multiple embryos in triplicate, while fold change values in C and D are means ± s.e.m. by genotype.