frontiers

Supplementary Material

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

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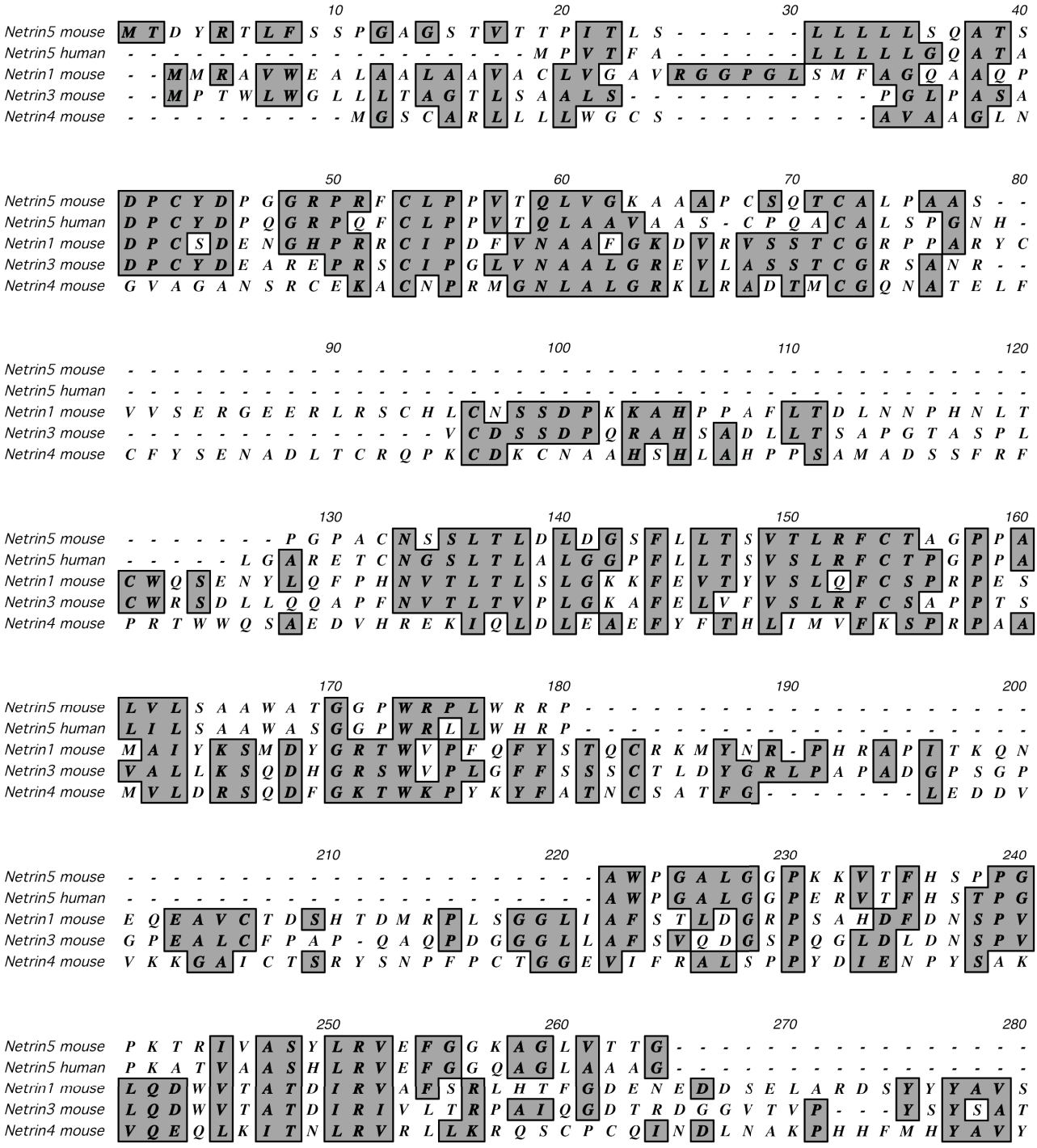
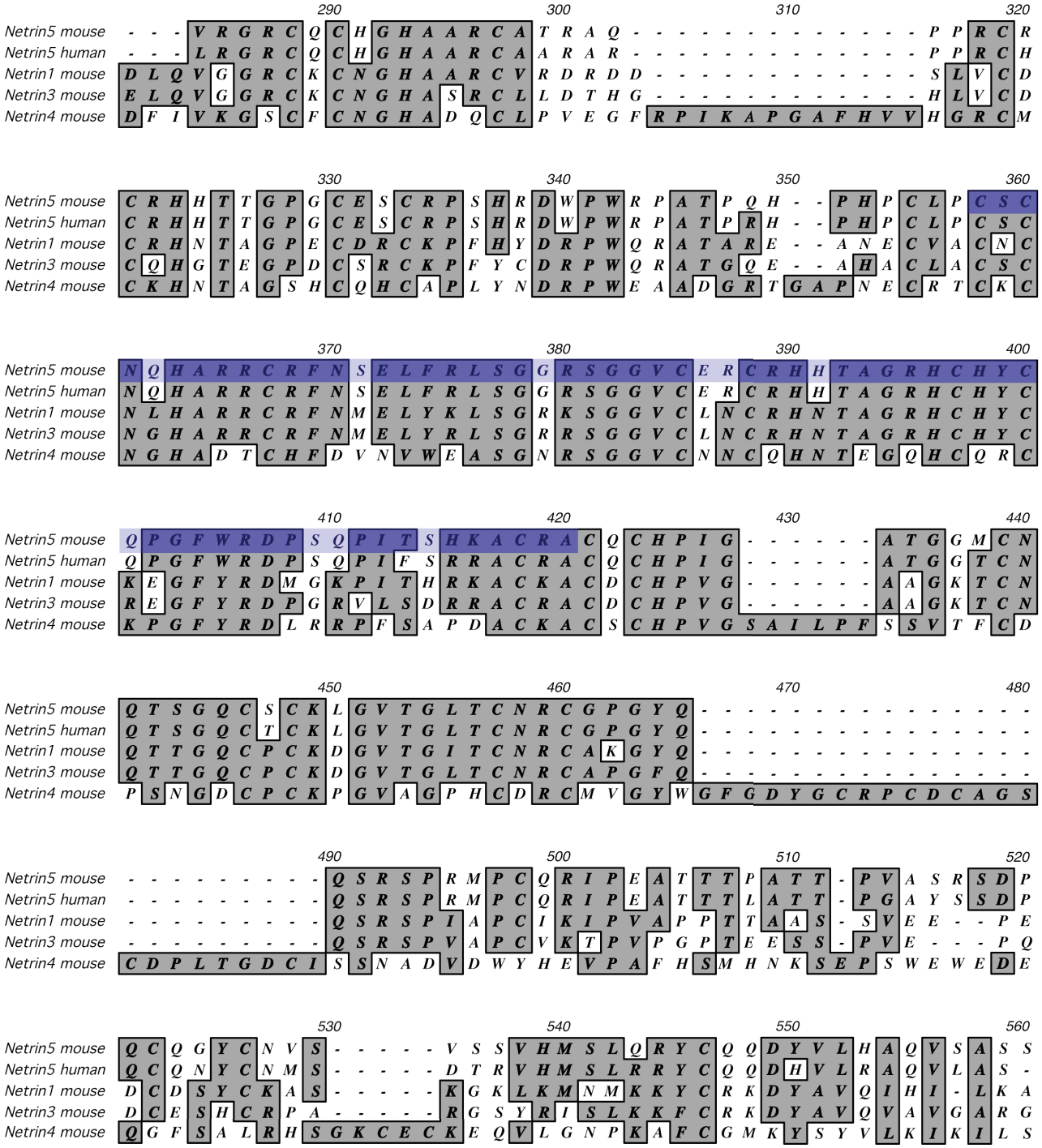
Supplementary Figures

A

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Netrin1 mouse D L Q V G G R C K C N G H A A R C V R D R D D - - - - - - - - - - - - - - - S L V C D
Netrin3 mouse E L Q V G G R C K C N G H A S R C L L D T H G - - - - - - - - - - - - - - - H L V C D
Netrin4 mouse D F I V K G S C F C N G H A D Q C L P V E G F R P I K A P G A F H V V H G R C M
330 340 350 360
Netrin5 mouse C R H H T T G P G C E S C R P S H R D W P W R P A T P Q H - - P H P C L P C S C
Netrin5 human C R H H T T G P G C E S C R P S H R D W P W R P A T P R H - - P H P C L P C S C
Netrin1 mouse C R 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 C
Netrin3 mouse C Q H G T E G P D C S R C K P F Y C D R P W Q R A T G Q E - - A H A C L A C S C
Netrin4 mouse C K H N T A G S H C Q H C 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 C
370 380 390 400
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Netrin5 human N Q H A R R C R F N S E L F R L S G G R S G G V C E R C R H H T A G R H C H Y C
Netrin1 mouse N L H A R R C R F N M E L Y K L S G R K S G G V C L N C R H N T A G R H C H Y C
Netrin3 mouse N G H A R R C R F N M E L Y R L S G R R S G G V C L N C R H N T A G R H C H Y C
Netrin4 mouse N G H A D T C H F D V N V W E A S G N R S G G V C N N C Q H N T E G Q H C Q R C
410 420 430 440
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Netrin3 mouse R E G F Y R D P G R V L S D R R A C 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 C K A C S C H P V G S A I L P F S S V T F C D
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Netrin3 mouse Q T T G Q C P C 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 C K P G V A G P H C D R C M V G Y W G F G D Y G C R P C D C A G S
490 500 510 520
Netrin5 mouse - - - - - - - - - Q S R S P R M P C Q R I P E A T T P A T T - P V A S R S D P
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Netrin3 mouse - - - - - - - - - Q S R S P V A P C V K T P V P G P T E E S S - P V E - - - P Q
Netrin4 mouse C D P L T G D C I S S N A D V D W Y H E V P A F H S M H N K S E P S W E W E D E
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Netrin1 mouse D C D S Y C K A S - - - - - K G K L K M N M K K Y C R K D Y A V Q I H I - L K A
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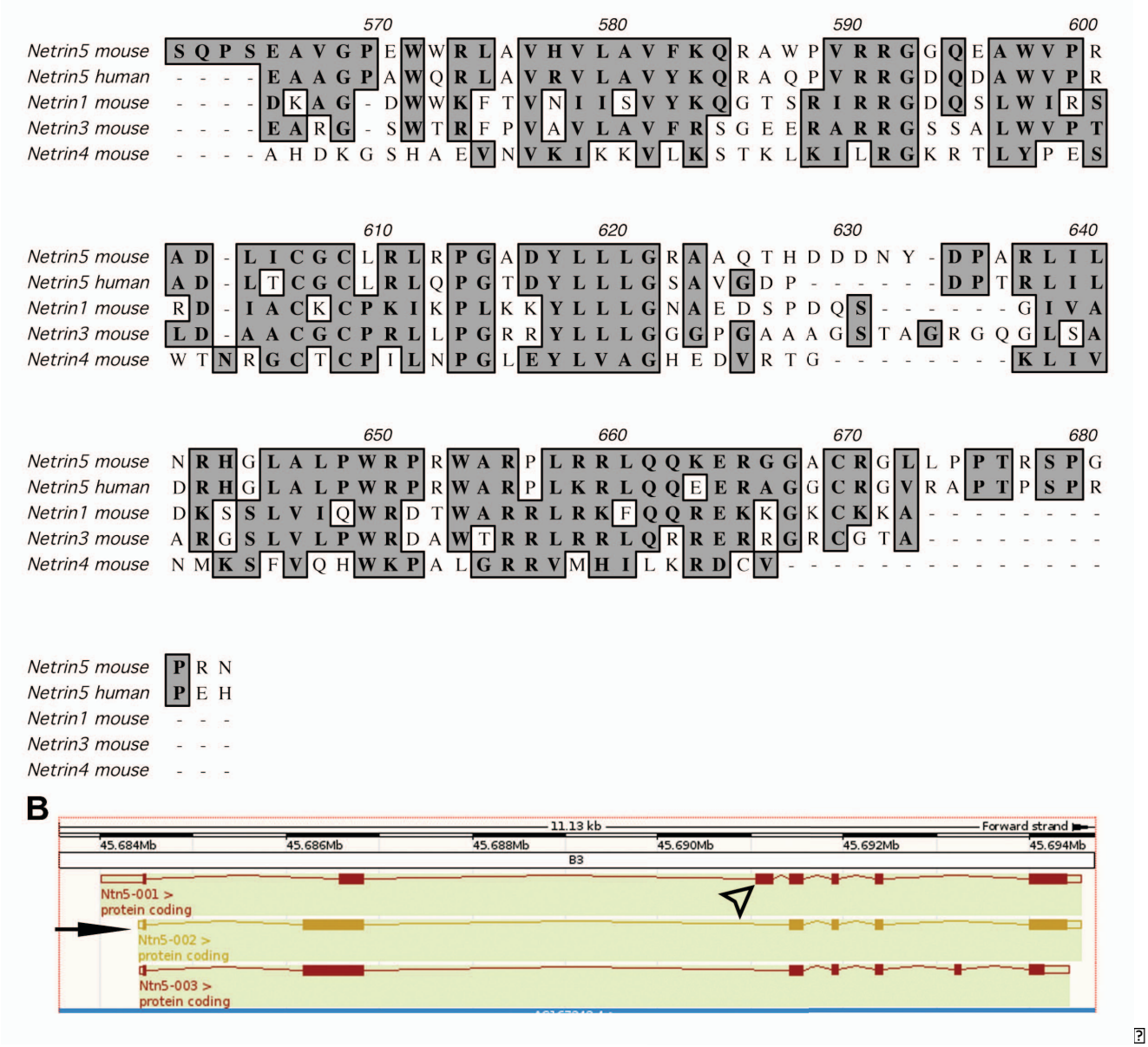

Figure S1: Netrin5 is a classic Netrin. Protein sequence alignment shows a high level of similarity between Netrin5 and other classic Netrins. Similar or identical residues are boxed. Sequence corresponding to the exon omitted in the short transcript is highlighted in blue (**A**). The gene structure 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.

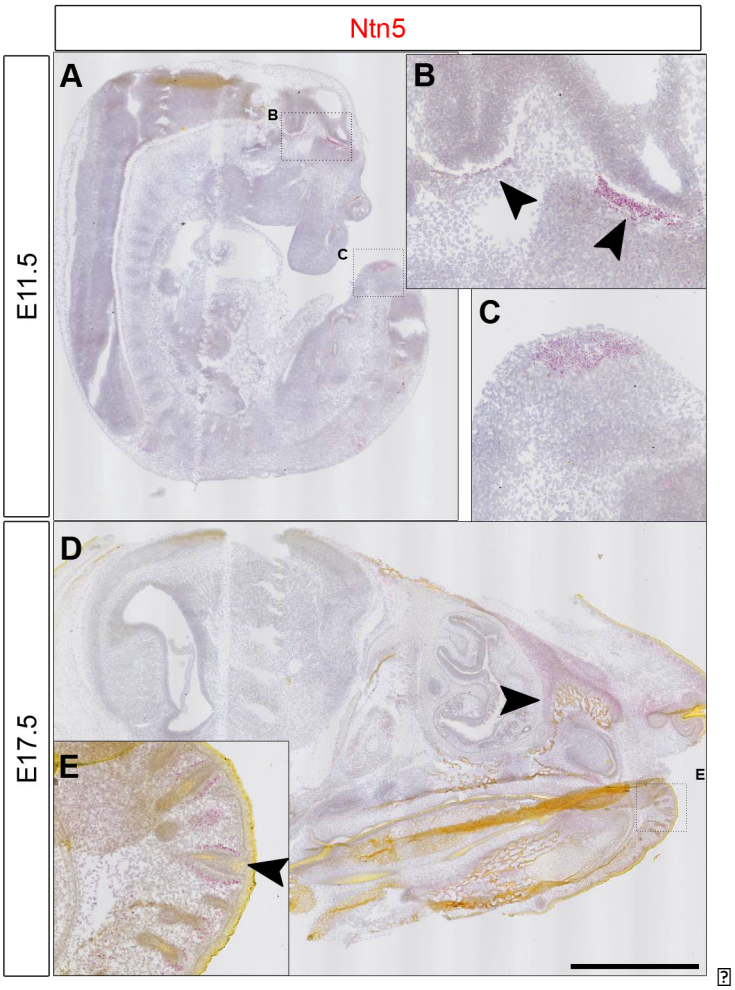

Figure S2: Other *Ntn5* expression. Netrin5 is expressed adjacent to the brain at E11.5 (**A**,**B**) and E17.5 (**D**). There was also expression associated with the olfactory system (**B**,**D** arrow), at the tail (**A**,**C**) and at the base of whiskers in the chin (**D**,**E**). Scale bar is 1.5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

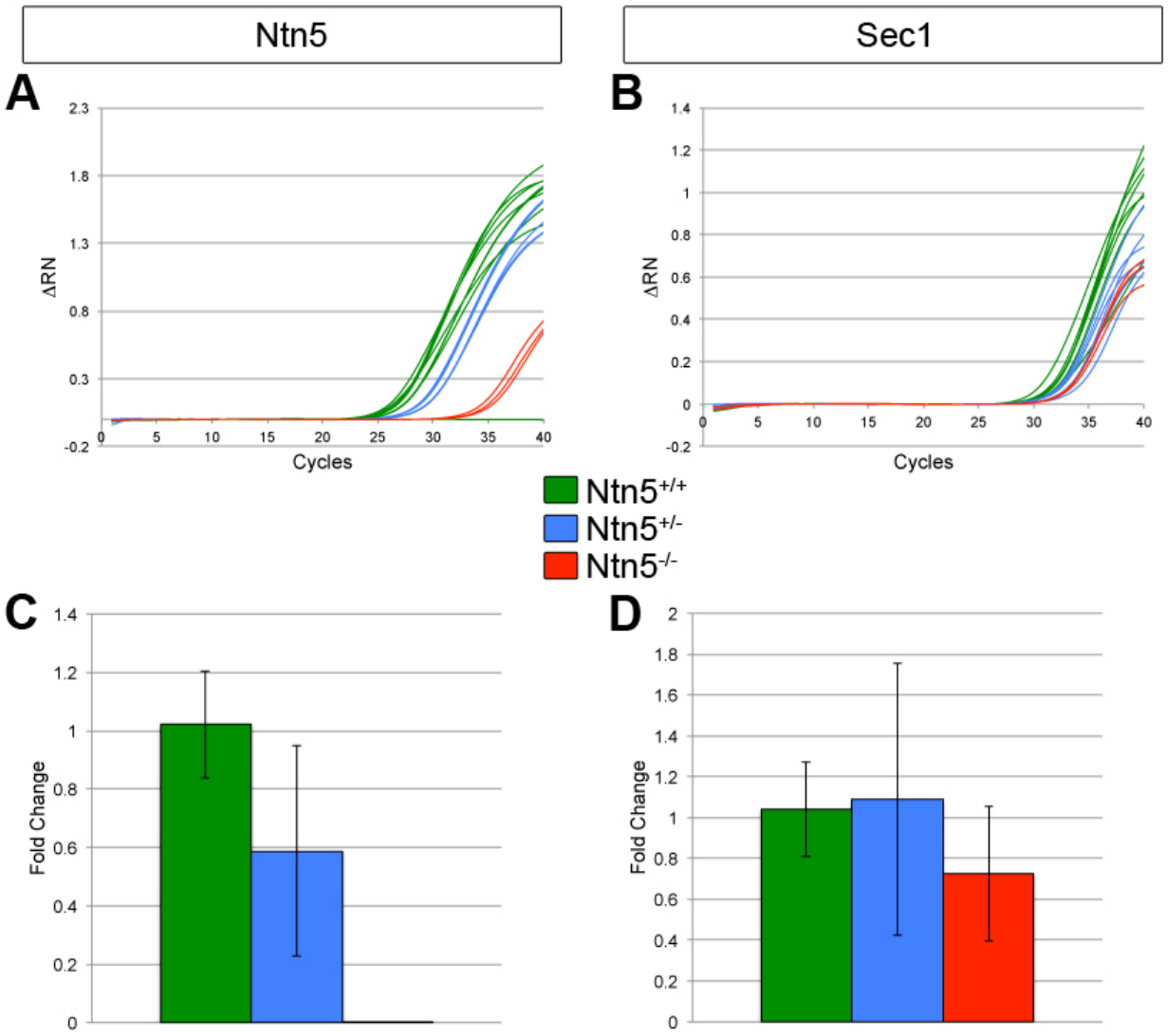

Figure S3: *Ntn5* expression abolished without disrupting *Sec1* expression in Ntn5$^{-/-}$ embryos. QPCR analysis of Ntn5 and Sec1 transcripts from E13.5 *Ntn5*$^{+/+}$, *Ntn5*$^{+/-}$, and *Ntn5*$^{-/-}$ whole embryos demonstrates that Ntn5 transcripts were effectively removed in null mutants (**A**,**C**), 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.