Cell Reports

Supplemental Information

miR-302 is required for timing of neural differentiation, neural tube closure, and embryonic viability

Ronald J. Parchem, Nicole Moore, Jennifer L. Fish, Jacqueline G. Parchem, Tarcio T. Braga, Archana Shenoy, Michael C. Oldham, John L.R. Rubenstein, Richard A. Schneider, Robert Blelloch

Figure 1 - figure supplement 1

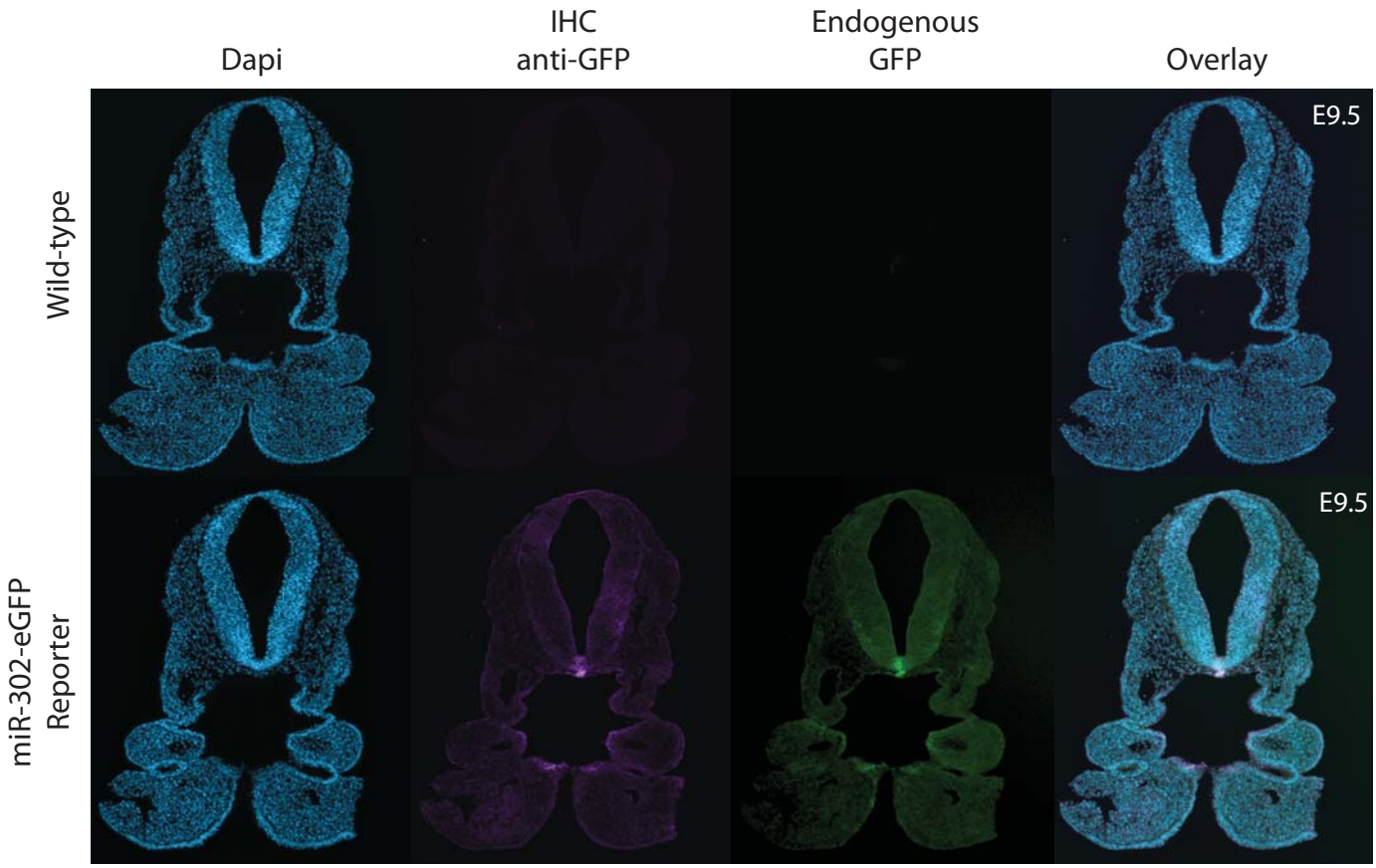
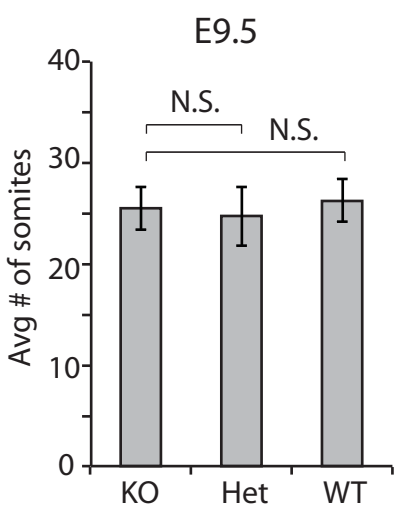


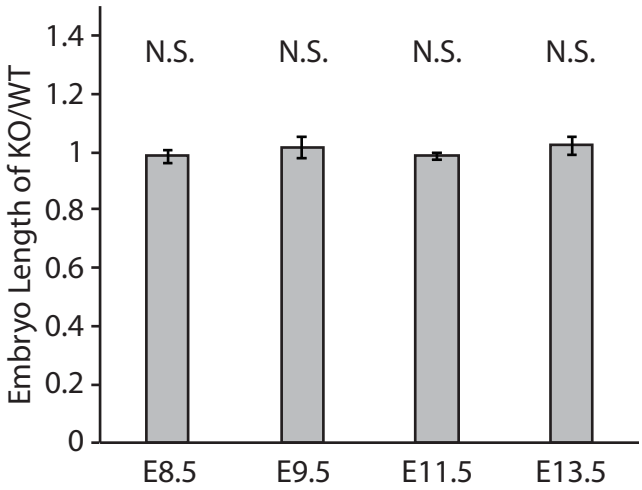
Figure 1-supplement figure 1: miR-302 reporter expression. Transverse sections of wild-type and miR-302-eGFP reporter mice at E9.5 stained for GFP expression.

Figure 2 - supplement figure 1

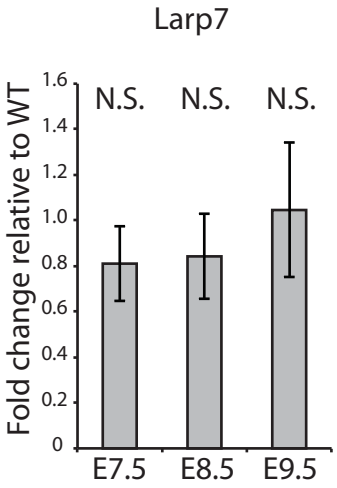
A



B



C



D

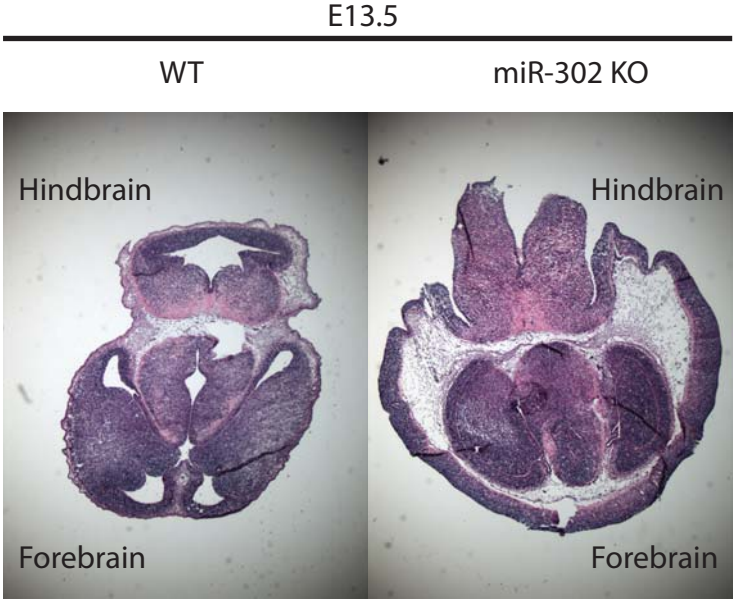


Figure 2-supplement figure 1: Phenotypic characterization of miR-302 knockout embryos. (**A**) Average somite number and genotype for litters analyzed at E9.5. (**B**) Average embryo length at indicated developmental stage relative to wildtype littermates. (n=20 litters) (**C**) RT-qPCR of Larp7 expression normalized to Rpl7. (n=3 embryos for each genotype and developmental stage). (**D**) H&E staining of transverse sections at E13.5 showing hindbrains of miR-302 knockout embryos are larger than wildtype.

Figure 3-supplement figure 1

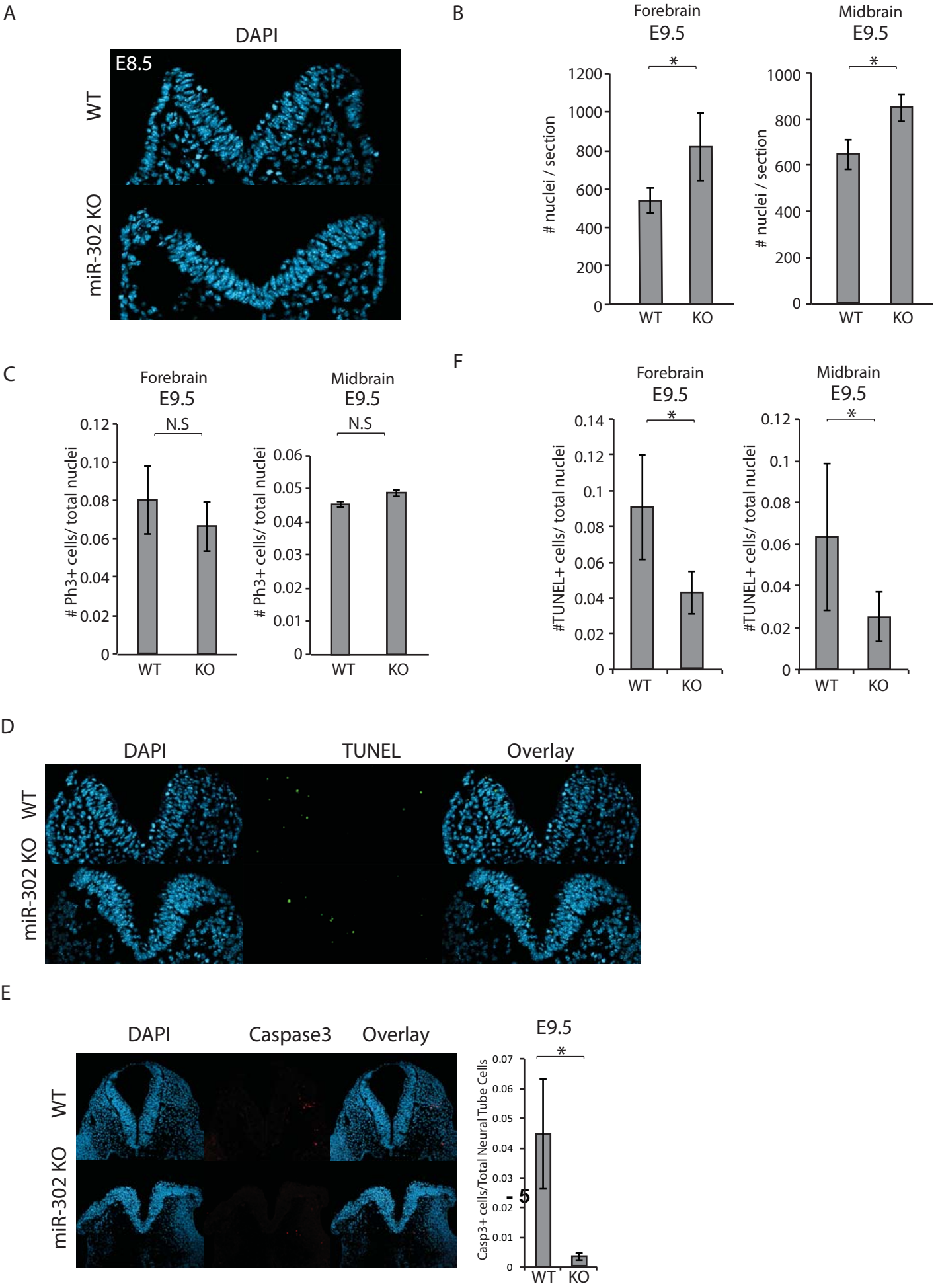


Figure 3-supplement figure 1: Cellular analysis of miR-302 knockout embryos. (**A**) Transverse section of developing hindbrain at E8.5 stained for DAPI to identify nuclei. (**B**) Thickening of neuroepithelium in miR-302 knockout embryos. Transverse sections of presumptive forebrain and midbrain at E9.5 were stained for DAPI to identify nuclei. (n=3 embryos, 6 sections/embryo) Error bars represent standard deviation. *p<0.05, **p<0.005 (**C**) Immunohistochemistry against phospho-histone H3 (pH3) to visualize cells in M-phase of the cell cycle. Quantification of pH3-positive cells was calculated as the percentage of pH3-positive cells out of total neuroepithelial cells at E9.5 in the forebrain and midbrain. Error bars represent standard deviation. (n=3 embryos, 6 sections/embryo). N.S. = not significant (**D**) TUNEL assay was used to identify apoptotic cells in transverse sections of presumptive hindbrain at E8.5. (**E**) Transverse section of presumptive hindbrain at E9.5 stained for Caspase3 to identify apoptotic cells. (n=3 embryos, 6 sections/ embryo) Error bars represent standard deviation. *p<0.05 (**F**) Transverse sections of presumptive forebrain and midbrain at E9.5 were stained for TUNEL to identify apoptotic cells. (n=3 embryos, 6 sections/embryo) Error bars represent standard deviation. *p<0.05

Figure 4-supplement figure 1

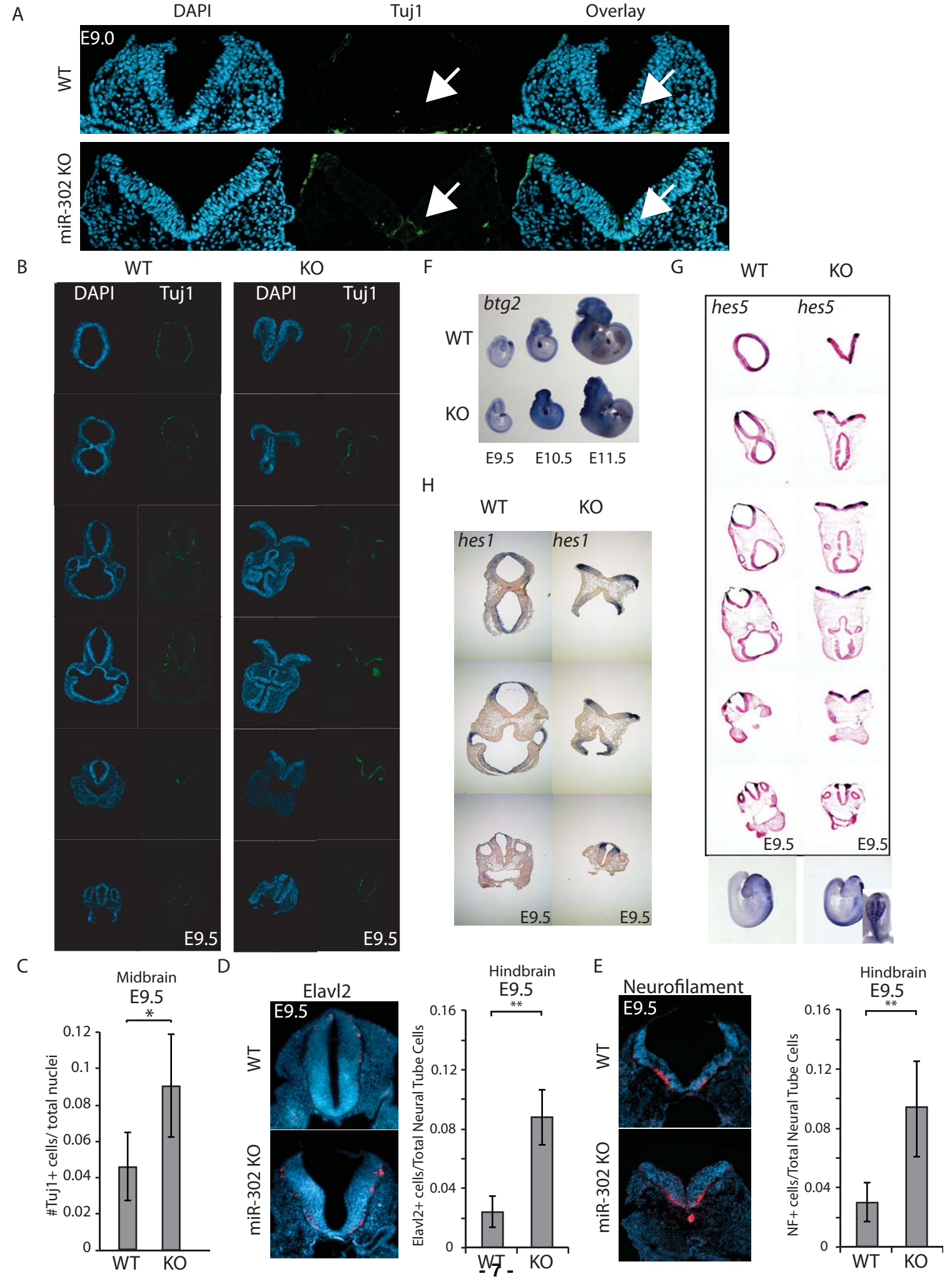


Figure 4-supplement figure 1: Differentiation defect in miR-302 knockout embryos. (**A**) Tuj1 immunohistochemistry was used to visualize post-mitotic neural cells at E9.0. (**B**) Tuj1 immunohistochemistry was used to visualize post-mitotic neural cells along the rostral-caudal axis. (**C**) Tuj1 immunohistochemistry to visualize post-mitotic neural cells in the midbrain at E9.5. Quantification of Tuj1+ cells was calculated as the percentage of Tuj1+ cells out of total neuroepithelial cells. Error bars represent standard deviation. (n=3 embryos, 6 sections/embryo). *$p<0.05$ (**D**) Elavl2 immunohistochemistry was used to visualize post-mitotic neural cells. Quantification of Elavl2+ cells was calculated as the percentage of Elavl2+ cells out of total neuroepithelial cells at E9.5. Error bars represent standard deviation. (n=3 embryos, 6 sections/embryo). **$p<0.005$ (**E**) Neurofilament immunohistochemistry was used to visualize post-mitotic neural cells. Quantification of NF+ cells was calculated as the percentage of NF+ cells out of total neuroepithelial cells at E9.5. Error bars represent standard deviation. (n=3 embryos, 6 sections/embryo). *$p<0.05$ (**F**) In situ hybridization staining for Btg2 from E9.5-E11.5 in wild-type and knockout embryos. (**G**) In situ hybridization staining for Hes5 followed by tranverse sectioning along the rostral-caudal axis in wild-type and knockout embryos at E9.5. (**H**) In situ hybridization staining for Hes1 followed by tranverse sectioning along the rostral-caudal axis in wild-type and knockout embryos at E9.5.

Figure 4-supplement figure 2

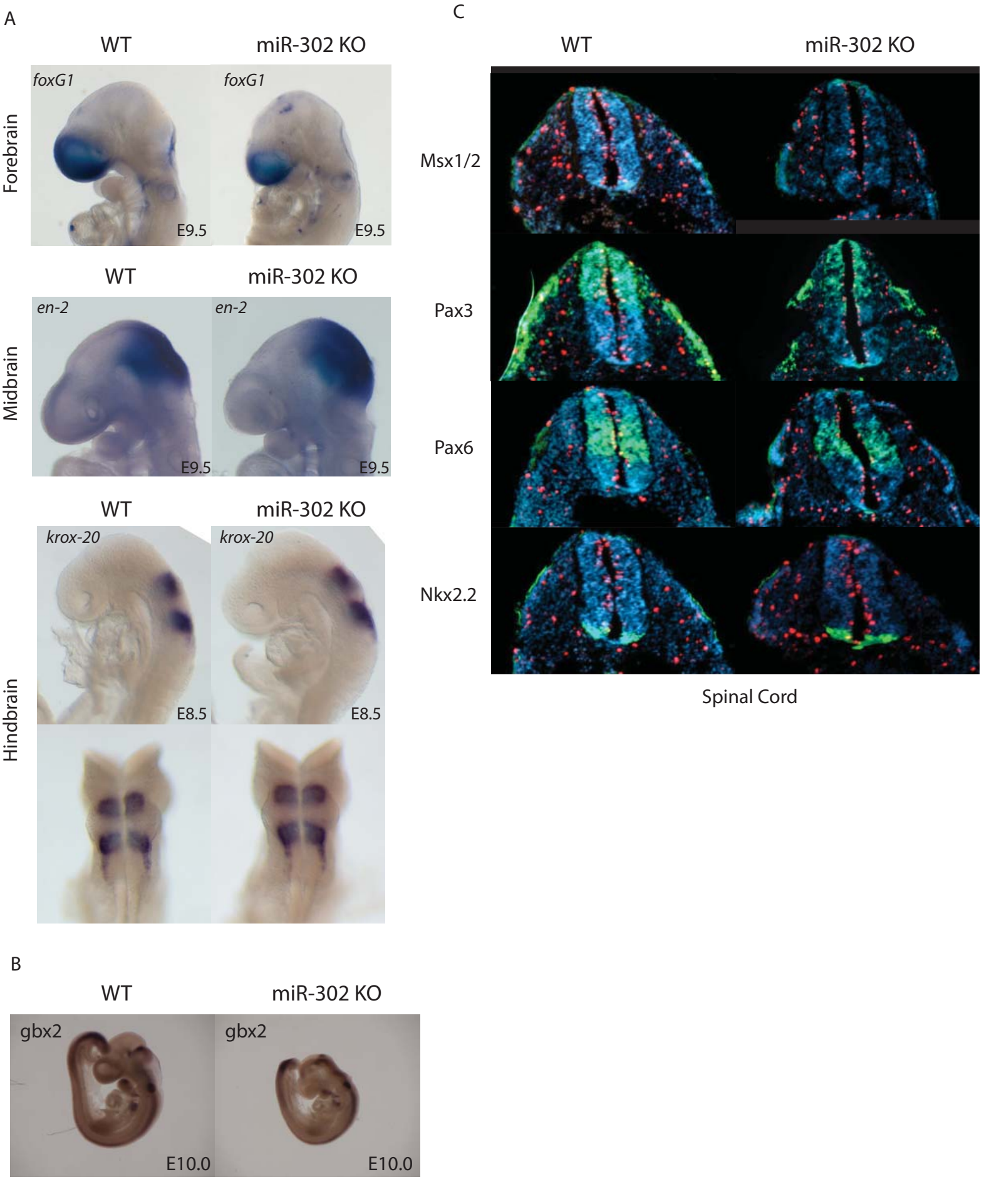


Figure 4-supplement figure 2: Patterning of miR-302 knockout embryos. (**A**) In situ hybridization staining for FoxG1, En-2, and Krox-20 in wild-type and knockout embryos at indicated developmental stages. (**B**)In situ hybridization staining for Gbx2 in wild-type and knockout embryos at E10.0. (**C**) Immunohistochemistry against Msx1/2, Pax3, Pax6, and Nkx2.2 in transverse sections of wild-type and knockout embryos just posterior to the hindbrain in the spinal cord.

Figure 6-supplement figure 1

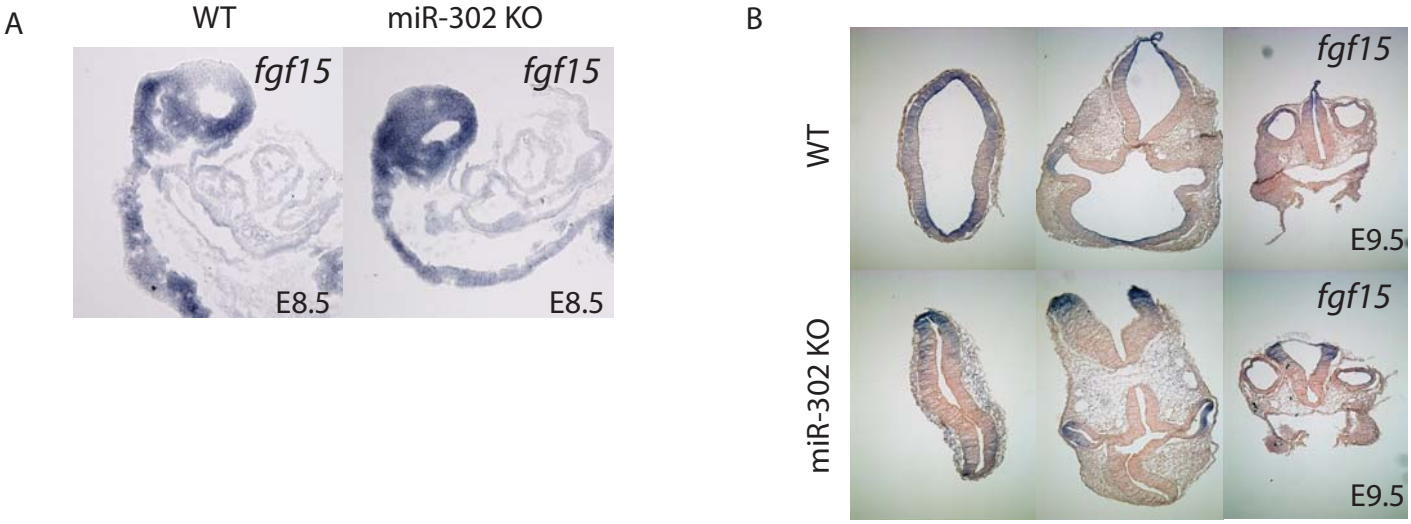


Figure 6-supplement figure 1: Overexpression of Fgf15 in miR-302 knockout embryos. (**A**) In situ hybridization staining for Fgf15 followed by saggital sectioning in wild-type and knockout embryos at E8.5. (**B**) In situ hybridization staining for Fgf15 followed by transverse sectioning in wild-type and knockout embryos at E9.5.

Supplemental Experimental Procedures:

Table of qPCR Primers

Name/ID	Primers	Primer Sequence
mmu-miR-302b-3p	302b	TAAGTGCTTCCATGTTTTAGTAG
mmu-miR-302c-3p	302c	AAGTGCTTCCATGTTTCAGTGG
mmu-miR-302a-3p	302a	TAAGTGCTTCCATHTTTTGGTGA
mmu-miR-302d-3p	302d	TAAGTGCTTCCATGTTTGAGTGT
mmu-miR-367-3p	367	AATTGCACTTTAGCAATGGTGA
mmu-miR-290a-5p	290a	ACTCAAACTATGGGGGCACTTT
mmu-miR-290b-3p	290b	AAGTGCCCCCATAGTTTGAGTA
mmu-miR-291a-5p	291a	CATCAAAGTGGAGGCCCTCTCT
mmu-miR-292-3p	292	AAAGTGCCGCCAGGTTTTGAGTGT
mmu-miR-292b-3p	292b	AAGAGCCCCCAGTTTGAGTAT
mmu-miR-291b-5p	291b	GATCAAAGTGGAGGCCCTCTCC
mmu-miR-293-3p	293	AGTGCCGCAGAGTTTGTAGTGT
mmu-miR-294-3p	294	AAAGTGCTTCCCTTTTGTGTGT
mmu-miR-295-3p	295	AAAGTGCTACTACTTTTGAGTCT
mmu-let-7c-5p	Let7c	TGAGGTAGTAGGTTGTATGGTT
mmu-let-7a-5p	Let7a	TGAGGTAGTAGGTTGTATAGTT
mmu-let-7i-5p	Let7i	TGAGGTAGTAGTTTGTGCTGTT
mmu-let-7g-5p	Let7g	TGAGGTAGTAGTTTGTACAGTT
mmu-let-7f-5p	Let7f	TGAGGTAGTAGATTGTATAGTT
mmu-let-7e-5p	Let7e	TGAGGTAGGAGGTTGTATAGTT
3'RACE Adaptor	3'RACE Adaptor	GCGAGCACAGAATTAATACGACTCACTATAGGT$_{12}$VN
sno202	sno202	GTACTTTTGAACCCTTTTCCATCTGATG
mRNA		
Uchl1	mmu_Uchl1_left	AGCTGGAATTTGAGGATGG
	mmu_Uchl1_right	CTCGAAACACTTGGCTCTATC
Msrb2	mmu_Msrb2_left	AGCAAGGACACAGGGTCTC
	mmu_Msrb2_right	CGCTTCAGTTCCTTTCTCTC
Tnfaip1	mmu_Tnfaip1_left	AGCCAGAGGCACTGAGAG
	mmu_Tnfaip1_right	CTGCAGGTAGATCACCACAC
Ednrb	mmu_Ednrb_left	ATCCGCACGAGTTGGTCTC
	mmu_Ednrb_right	CATGTTACAGCTTGCTCCTGTG
Fgf15	mmu_Fgf15_left_001	ATATACGGGCTGATTCGCTAC
	mmu_Fgf15_right_001	GCCTAAACAGTCCATTTCCTC
Elavl2	mmu_Elavl2_left	CGCAGCAGCAGGTAATTG
	mmu_Elavl2_right	GTCTTGCTGTCCTCTGTGTTC
Stau2	mmu_Stau2_left	TTCAGATAAATCAGCTTCTCTCC
	mmu_Stau2_right	CTATGGAAACGGGCTAACTC
Cul1	mmu_Cul1_left	AAATCTTCTTAAGGATGGAG
	mmu_Cul1_right	GTGTAGAACTTCAGGACACTC

Lamp2	mmu_Lamp2_left	CTGTTCCTAGGAGCCGTTC
	mmu_Lamp2_right	GCAAGTACCCTTTGAATCTGTC
Med21	mmu_Med21_left	CTGTGAACTCGCTTGCAG
	mmu_Med21_right	CTGTAGGATTGGCTGGTTG
Neurod4	mmu_Neurod4_left	CCCGGGAAAGAGAATCTATAC
	mmu_Neurod4_right	TCTCTCTTGCTCCTTCATCTC
Dazap2	mmu_Dazap2_left	GAACAGCAAAGGTCAATATCC
	mmu_Dazap2_right	CAGGTGGAGCATCAGTATAGG
Rpl7	mmu_Rpl7_left	CTACTTGGGCGGAGAGAG
	mmu_Rpl7_right	CAACTCTGCGAAATTCCTTC
actin (ACTB)	mmu_actin_left	CTAGGCACCAGGGTGTGATG
	mmu_actin_right	GGCCTCGTCACCCACATAG
GAPDH (GAPD)	mmu_GAPDH_left	CAGGAGAGTGTTTCCTCGTC
	mmu_GAPDH_right	TTCACACCGACCTTCACC