

Supplementary figures

Table 1: RT-qPCR primer sequences.

Gene (RefSeq mRNA transcript ID)	Oligonucleotide primer sequence (5'-3')		Primer efficiency (%)
	Forward	Reverse	
<i>ActB</i> (NM_007393)	GGCTGTATCCCCTCCATCG	CCAGTTGGTAATGCCATGT	90%
<i>Aff2</i> (NM_008032)	CTACCACAACCACTACCACTA	CCAGGTGACTGCTATCCATAA	168%
<i>Asx13</i> (NM_001167777)	CTGCTATTCCAGCGTCAGATAC	GCCTCAACTGTTCTCTCATAC	73%
<i>Bmpr2</i> (NM_007561)	GGCACATAGGTCCCAAGAAA	CTGTTGCTCTCGTCTCAGTAAA	76%
<i>Cecr5</i> (NM_144815)	CTCAGGAGAAGGGATGGATTTTC	CACTGAGGCACAGAGGTAAAG	112%
<i>Chmp1a</i> (NM_145606)	GAGAGTAGCTGCAGTCTCGG	CCAGCTCCCCATCCCTACTA	104%
<i>Col9a1</i> (NM_007740)	GATTGGCGTGGAAGAACAAG	CAAAGCCATCCGCATCAATC	92%
<i>Dicer1</i> (NM_148948)	CTCGAGATCCTGCTGCGCAAATA	GAATCACTCCAGGACACGTAA	137%
<i>Eif2s3y</i> (NM_012011)	TGCTCCAGGTGGTCTTATTG	ACCTGCCCCAACCATTCTATC	102%
<i>Fmc1</i> (NM_025363)	TTCCAAGCTGCCACCTATC	CAACTGGAGGCCCACTAAA	90%
<i>Rpl37</i> (NM_026069)	CCAAGCGCAAGAGGAAGTATAA	CATGTCTGAATCTGCGGTAGAC	84%
<i>Sox9</i> (NM_011448)	GGCAAGCTCTGGAGGCTG	CCTCCACGAAGGGTCTCTTCT	98%
<i>Xist</i> (NR_001463)	GCCCAAAGGGACAAACAATC	GTAGCGAGGACTTGAAGAGAAG	134%

Table 2: miRNA RT-qPCR primer sequences.

miRNA	Forward primer sequence (5'-3')	Primer efficiency (%)	E-val
<i>cel-miR-39</i>	TCACCGGGTGTAATCAGCTTG	57%	1.57
<i>mmu-miR-9-3p</i>	TAAGCTAGATAACCGAAAGTAAAAAA	70%	1.7
<i>mmu-miR-10b-5p</i>	CCTGTAGAACCGAATTTGTGA	218%	3.18
<i>mmu-miR-19a-3p</i>	TGTGCAAATCTATGCAAACTGA	320%	4.2
<i>mmu-miR-101a-3p</i>	TACAGTACTGTGATAACTGAAAAAAA	68%	1.68
<i>mmu-miR-130b-3p</i>	CAGTGCAATGATGAAAGGGCAT	180%	2.80
<i>mmu-miR-199a-5p</i>	CCCAGTGTTCCAGACTACCTGTTT	177%	2.77
<i>mmu-miR-200c-3p</i>	TAATACTGCCGGGTAATGATGGA	266%	3.66
<i>mmu-miR-205-5p</i>	TCCTTCATTCCACCGGAGTCTGAAAA	153%	2.53
<i>mmu-miR-206-3p</i>	TGGAATGTAAGGAAGTGTGTGG	182%	2.82
<i>mmu-miR-409-5p</i>	AGGTTACCCGAGCAACTTTGCAT	157%	2.57
<i>mmu-miR-872-5p</i>	AGGTTACTTGTTAGTTCAGGAAAA	317%	4.17

Table 3: ISH probe primer sequences.

Gene name	Oligonucleotide primers (5'-3')		Probe conc.	Hybe temp (°C)	Staining time
	Forward	Reverse			
<i>Aff2</i>	AAATCCCTGAGAAGAAGCA G	TGACATTTCTGCGACGTG	1:100	60	2.5 h
<i>Dicer1</i>	AAGCAGCCAACAAAAGAG	CCTGAGGCTGGTTAGCTTT G	1:100	60	2.5 h
<i>Xist</i>	AGGAAAAGATGGCGGCTC AA	CAGCCTCGGTCTCTCGAATC	1:100	60	2.5 h

Table 4: LNA probe sequences.

miRNA name	Probe sequence (5'-3')	Probe conc (nM)	Hybridisation temperature (°C)	Staining time
<i>miR-10a-5p</i>	/5DigN/ACA AAT TCG GAT CTA CAG GGT A/3DigN/	20	60	1.5 h
<i>miR-101a-3p</i>	5DigN/CTTCAG+TTATCA+CAGTAC+TGTA	25	48	O/N
<i>miR-200b-3p</i>	/5DigN/TCAT+CATTACC+AGGCAG+ATTA	25	52	2 h

A)

Sample name	Forward reads			Reverse reads		
	Raw	Trimmed	Mapped	Raw	Trimmed	Mapped
M1	20475910	20303997	14526548 (72%)	20475910	20303997	16020276 (79%)
M2	14385179	14257133	11451283 (80%)	14385179	14257133	12314718 (86%)
M3	23050900	22842935	18406586 (80%)	23050900	22842935	19786418 (87%)
F1	19305369	19094665	13868666 (73%)	19305369	19094665	15822849 (83%)
F2	15791013	15517122	10969635 (71%)	15791013	15517122	12686292 (82%)
F3	21756401	21482279	16889747 (79%)	21756401	21482279	18312524 (85%)



Figure 1: RNA-seq mapping statistics and read quality. **A)** Read count data for each sequenced sample in raw file, in the output from trimming and QC, and following mapping to the mm9 reference genome for both forward and reverse sequences. **B)** MultiQC plot of aggregated phred scores for all samples across the 125 bp read length.

A)

Sample name	Raw read counts-	Trimmed & filtered read counts	Mapped read counts
M1	35384650	21987252	21795844 (99.13%)
M2	67476605	48522046	48329540 (99.60%)
M3	64680459	30720891	30300908 (98.63%)
F1	31385476	17074620	16962741 (99.34%)
F2	84664948	65820211	65573574 (99.63%)
F3	89442033	45110149	44841628 (99.40%)

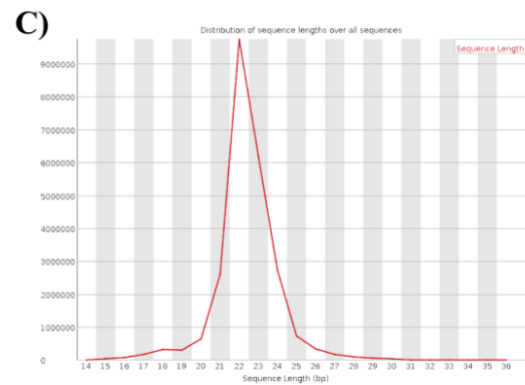
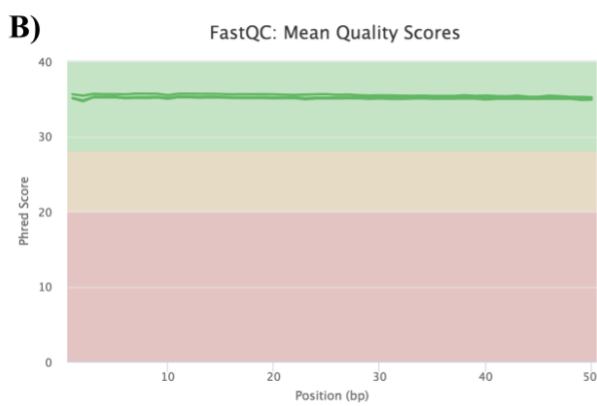


Figure 2: Small RNA-seq mapping statistics and read quality. **A)** Read count data for each sequenced sample in the raw file, in the output from trimming and QC, and following mapping to the mm9 reference genome, with the percentage of trimmed reads successfully mapped in brackets. **B)** FastQC plot of a representative sample showing phred score across the 50 bp untrimmed read. **C)** Read length distribution plot for a representative sample showing that read lengths peak at ~22 bp.

Table 5: Top 10 miRNAs in small RNA-seq data by combined (n = 6) read count.

Mature miRNA	Total read counts	Literature search
<i>mmu-miR-9-5p</i>	33824856	Highly expressed during neurogenesis to regulate various aspects of vertebrate brain development (135).
<i>mmu-miR-125b-5p</i>	18302138	Expressed in the mouse embryonic nervous system from stage E12 (136).
<i>mmu-miR-92a-3p</i>	8007628	Specifically expressed in glial cells in the neonatal rat brain (137).
<i>mmu-miR-181a-5p</i>	7788531	<i>miR-181</i> family members are highly expressed in the cortex and involved with synaptogenesis. Increased expression noted in autism animal models and patients (138).
<i>mmu-miR-99a-5p</i>	5823098	High expression in human NSC cultures (139) and associated with cell cycle regulation (140).
<i>mmu-let-7c-5p</i>	5780019	Expression levels continuously rise from E12.0 in embryonic mouse brains and impact stem cell behavior (141).
<i>mmu-miR-9-3p</i>	5752114	The sister strand of <i>mmu-miR-9-5p</i> has also been implicated in various facets of neural cell development (103).
<i>mmu-miR-99b-5p</i>	5144303	See <i>mmu-miR-99a-5p</i> .
<i>mmu-miR-26a-5p</i>	4807191	Various roles in mouse brain development including as a component of a key regulatory circuit for neural progenitor proliferation (142).
<i>mmu-let-7a-5p</i>	4735868	See <i>mmu-let-7c-5p</i> .

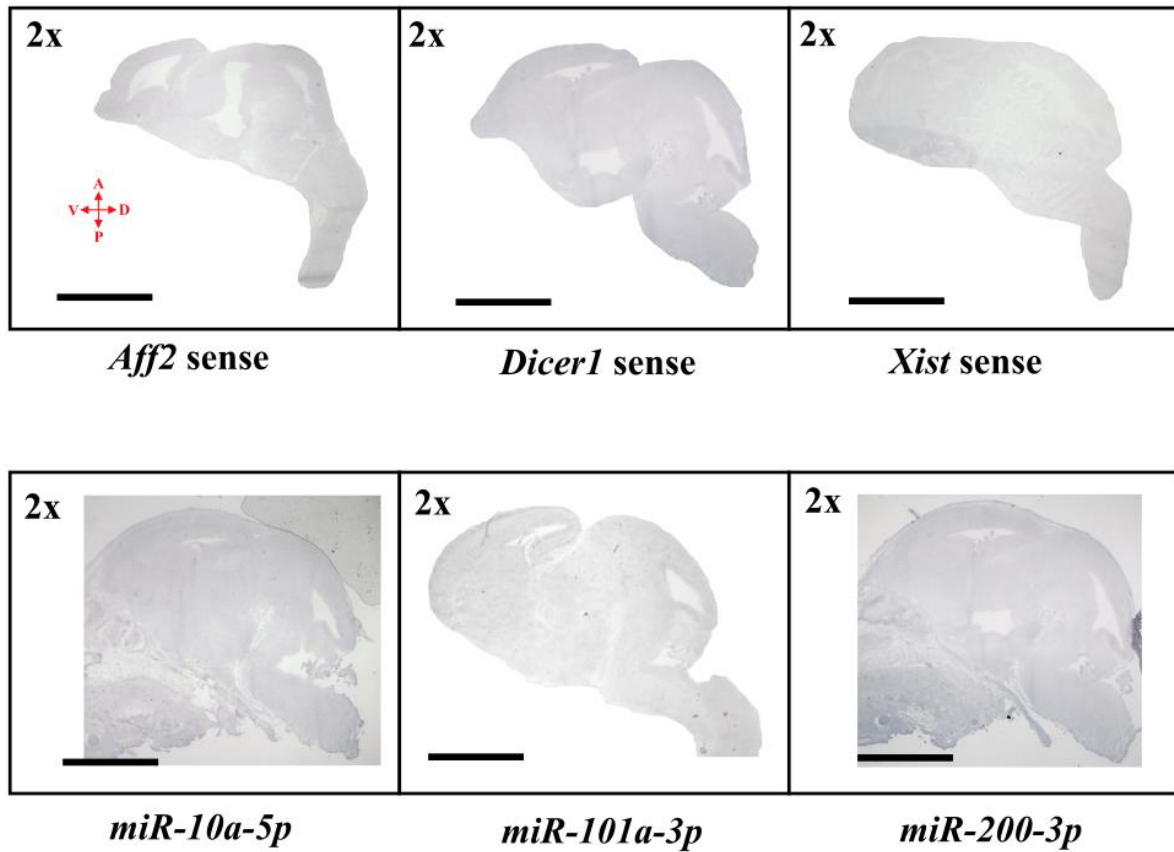


Figure 3: ISH negative controls. **Representative negative control ISH images for 8 different probes in sagittal sections of E15.5 mouse brains.** All are 2x magnification images, scale bar = 1000 μ m.

