Supporting Information

Multiple knockout mouse and embryonic stem cell models reveal the role of *miR-124a* in neuronal maturation

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Material included:

Fig. S6: The genomic DNA sequences of mutant alleles in *miR-124a-1/2/3* TKO ES clones #21, #71, and #93.
Fig. S7: Phase-contrast view of *miR-124a-1/2/3* TKO cells during induced neuronal differentiation.
Fig. S8: Gene expression analysis of glial markers in neuronal differentiation-induced *miR-124a-1/2/3* TKO cells.
Table S1: Sequences of DNA oligonucleotides (5’ → 3’)

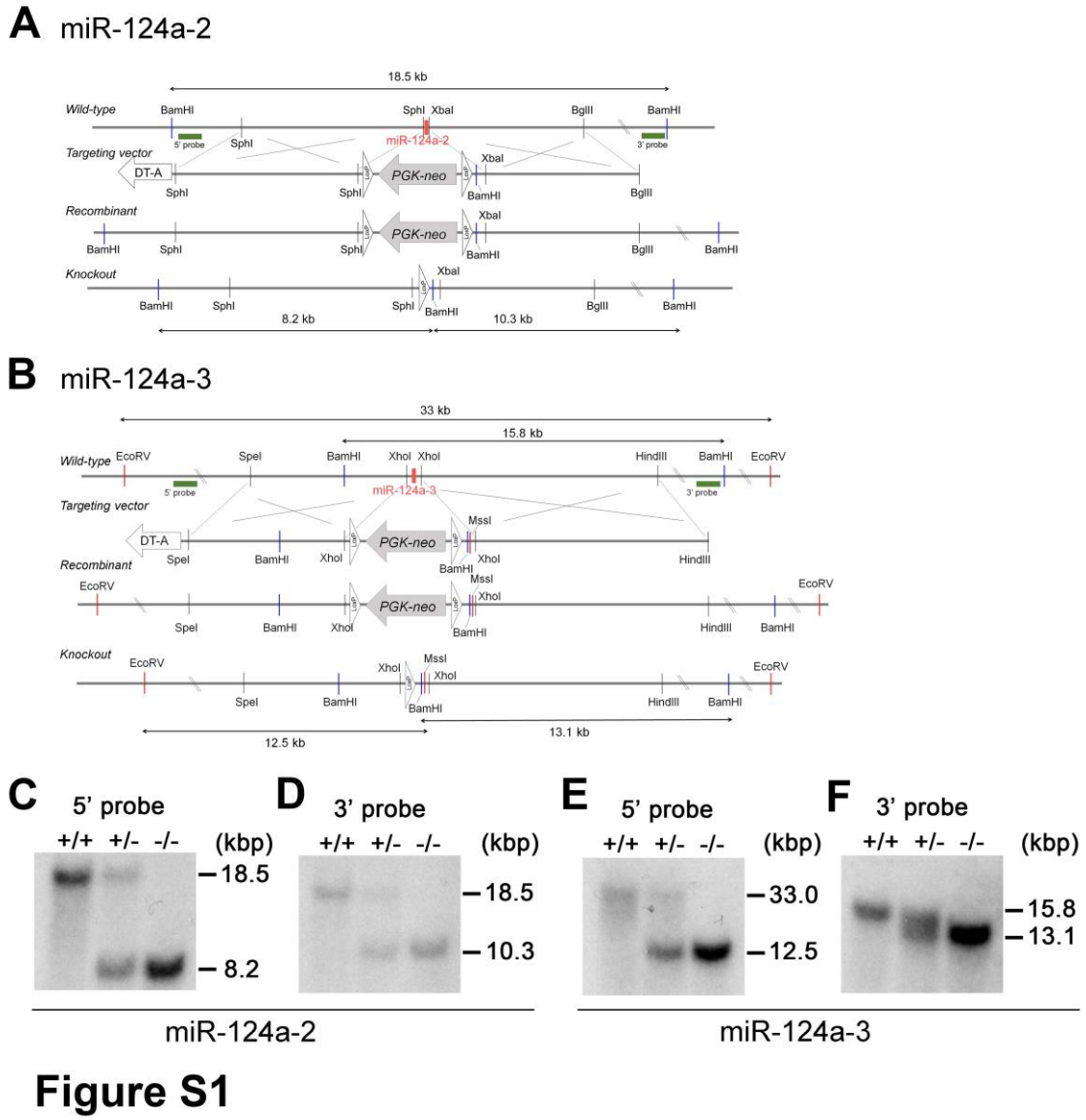

Figure S1

Figure S1. Generation of *miR-124a-2*$^{-/-}$ *and -3*$^{-/-}$ mice.

(A, B) Diagrams of the targeting vectors and the *miR-124a-2*$^{-/-}$ *and -3*$^{-/-}$ alleles. The *miR-124a-2*$^{-/-}$ (A) and -*3*$^{-/-}$ (B) alleles are shown. Genomic regions encoding *miR-124a-2* and -*3* precursors are replaced by floxed *PGK-neo* cassettes.

(C-F) Southern blot analysis of genomic structure of *miR-124a-2* and -*3* loci. BamHI- (C, D, F) or EcoRV/MssI-digested (E) genomic DNA was hybridized with 5′(C, E) and 3′

probes (D, F) for *miR-124a-2* (C, D) and *-3* loci (E, F). The 5′ probe of *miR-124a-2* detected 18.5-kb WT and 8.2-kb mutant bands (C). The 3′ probe of *miR-124a-2* detected 18.5-kb WT and 10.3-kb mutant bands (D). The 5′ probe of *miR-124a-3* detected 33.0-kb WT and 12.5-kb mutant bands (E). The 3′ probe of *miR-124a-3* detected 15.8-kb WT and 13.1-kb mutant bands (F).

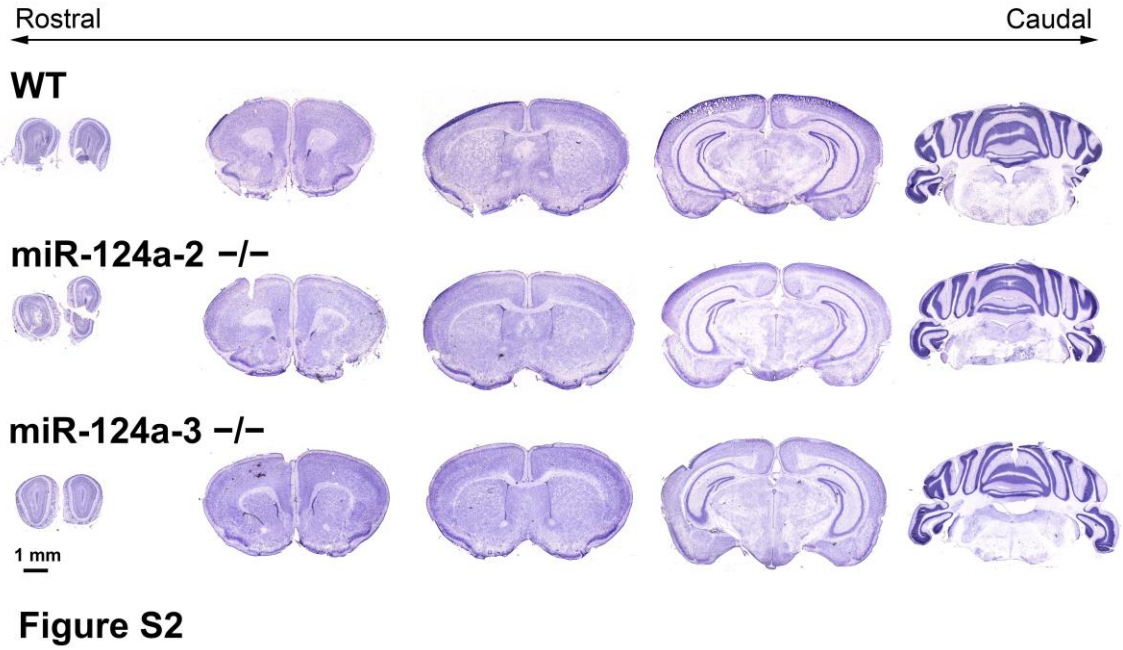

Figure S2

Figure S2. Coronal sections from the WT control, *miR-124a-2*$^{-/-}$, and -*3*$^{-/-}$ brains.

Nissl staining of serial brain sections arranged from rostral to caudal of the WT control, *miR-124a-2*$^{-/-}$, and -*3*$^{-/-}$ mice at 2M. Scale bar, 1 mm.

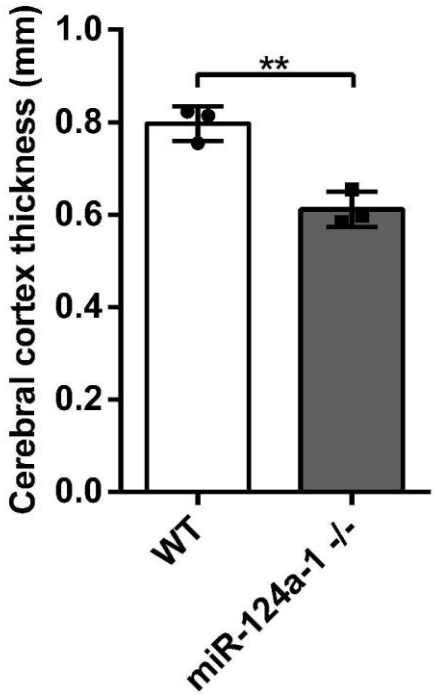

Figure S3

Figure S3. Thickness of the cerebral cortex in the WT control and *miR-124a-1*$^{-/-}$ mouse brains.

The thickness of the cerebral cortex in the WT control and *miR-124a-1*$^{-/-}$ brains was measured (n = 3 per each genotype). Data are presented as the mean ± SD. $^{**}p < 0.01$ (unpaired t-test).

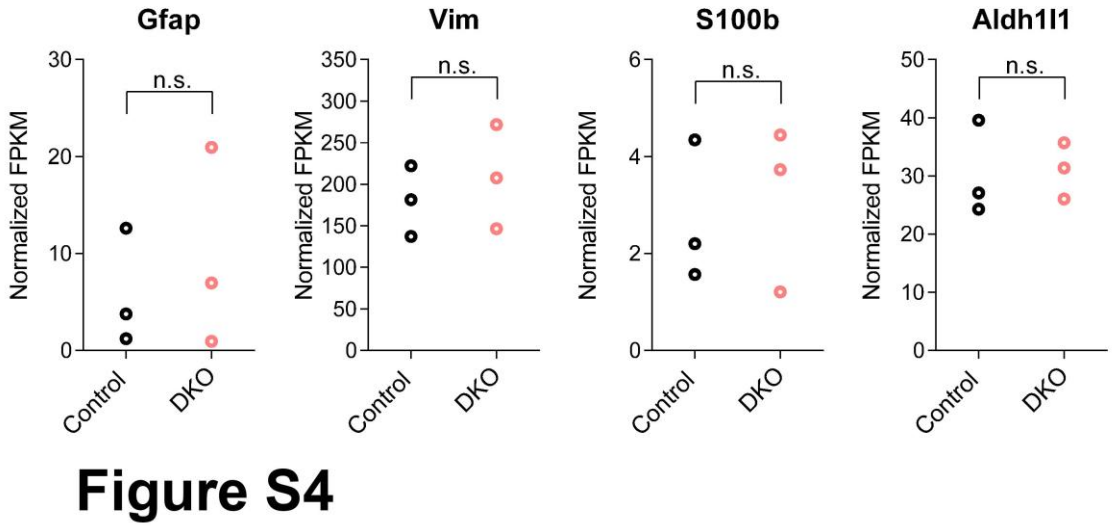

Figure S4

Figure S4. Gene expression analysis of glial markers in the *miR-124a-1/2* DKO mouse brain.

Gene expression levels of *Gfap*, *Vim*, *S100b*, and *Aldh1l1* (radial glia and/or astrocyte markers) in the control and *miR-124a-1/2* DKO brains at E17.5. n.s., not significant (unpaired t-test), n = 3 per genotype.

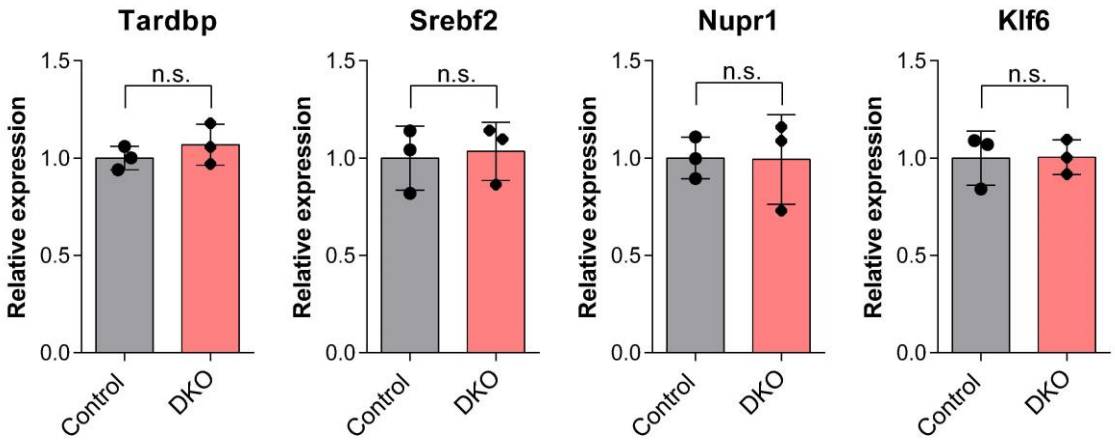

Figure S5

Figure S5. Expression levels of genes encoding transcription regulators in the *miR-124a-1/2* DKO mouse brain.

Quantitative RT-PCR analysis of the *Tardbp*, *Srebf2*, *Nupr1*, and *Klf6* mRNAs in the control and *miR-124a-1/2* DKO brains at E17.5. Data are presented as the mean ± SD. n.s., not significant (unpaired t-test), n = 3 per genotype.

Figure S6

Figure S6. The genomic DNA sequences of mutant alleles in *miR-124a-1/2/3* TKO ES clones #21, #71, and #93.

The mature *miR-124a* sequence is indicated in red. Deletion and insertion are shown in blue and green, respectively. Biallelic mutations in all three *miR-124a* genes were identified in clones #21, #71, and #93.

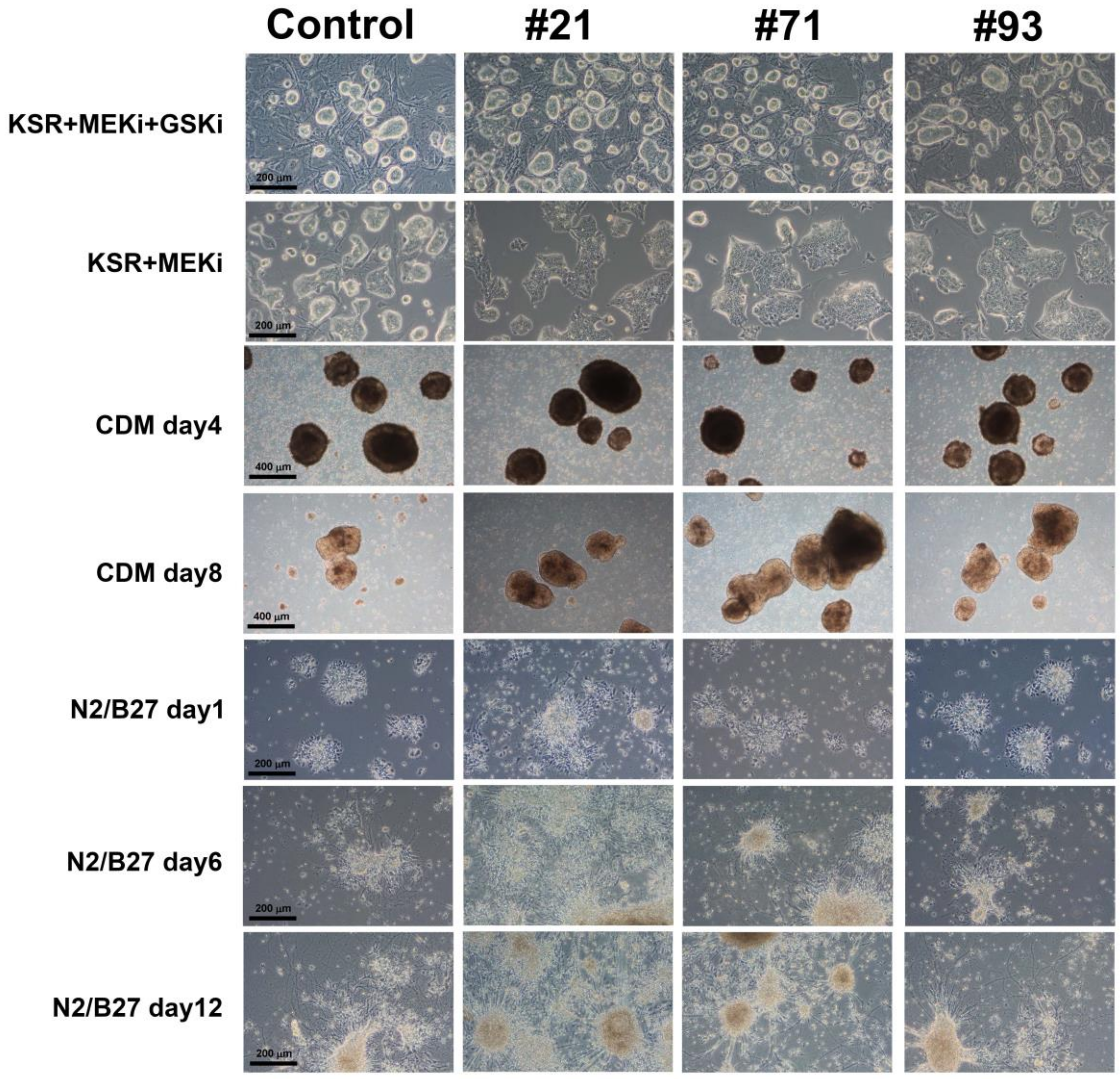

Figure S7

Figure S7. Phase-contrast view of *miR-124a-1/2/3* TKO cells during induced neuronal differentiation.

Neuronal differentiation from the control and *miR-124a-1/2/3* TKO ES cells was induced as shown in Figure 4B. 2i, MEKi and GSKi.

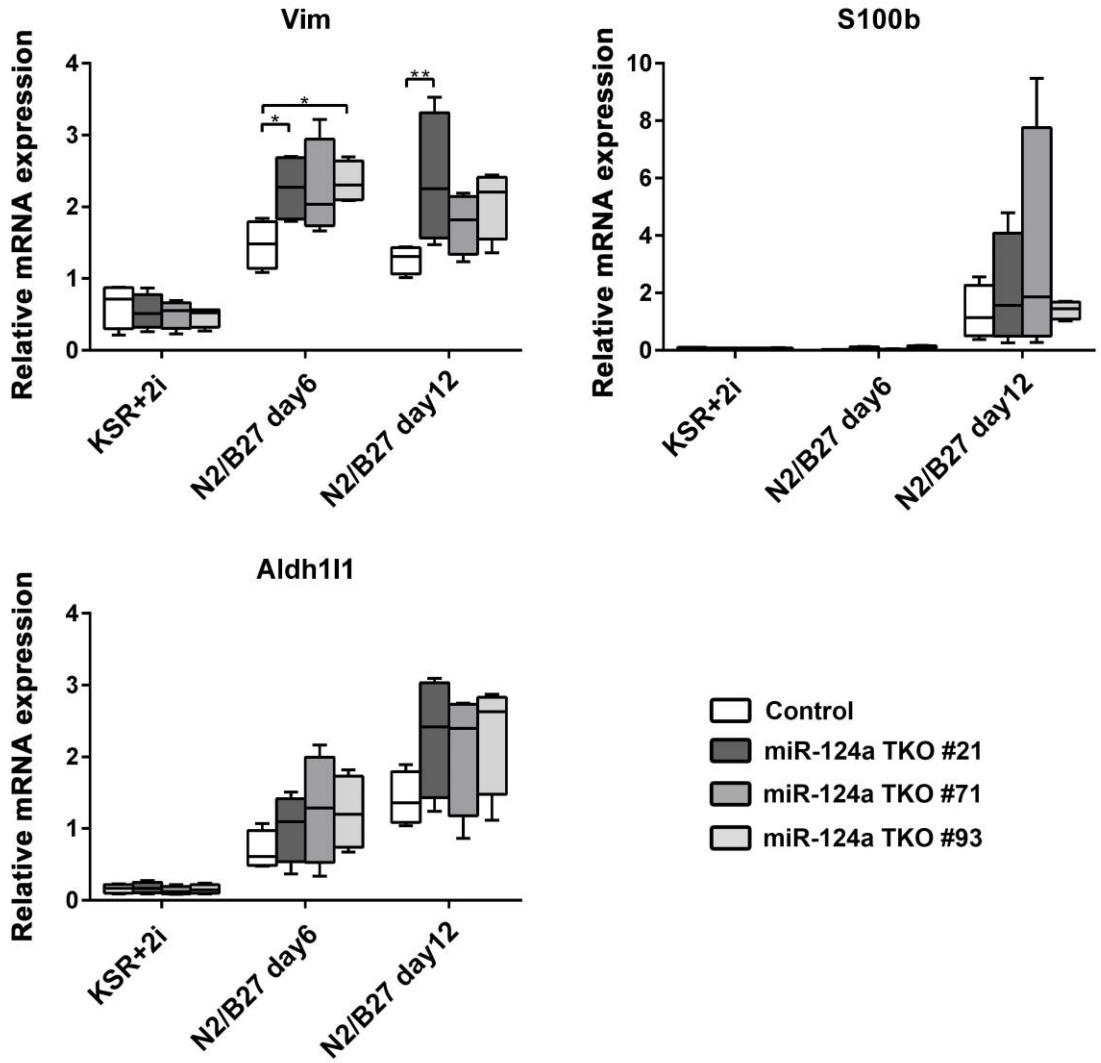

Figure S8

Figure S8. Gene expression analysis of glial markers in neuronal differentiation-induced *miR-124a-1/2/3* TKO cells.

Expression levels of genes for glial markers (*Vim*, *S100b*, and *Aldh1l1*) in *miR-124a-1/2/3* TKO cells at the indicated time points were analyzed. RNA was purified from cells cultured in ES medium (KSR+2i), and neuronal differentiation medium (N2/B27) for 6 or 12 days. Box–whisker plots present the median (center line), ±1.5 interquartile range

(box) and minimal and maximal values (whiskers). $^{*}p < 0.05$, $^{**}p < 0.01$ (two-way ANOVA followed by Bonferroni test), n = 4 per cell line. 2i, MEKi and GSKi.

Table S1. Sequences of DNA oligonucleotides (5' → 3')

Southern blotting probe	Sequence	Reference
miR-124a-2 5probe 51	GAAGTTGCACCTCTCCAGTGTTCCAGTG	
miR-124a-2 5probe 31	GTCACACTGATAACATCCCTCAGTGCTC	
miR-124a-2 3probe 51	GCTTCTACCCTGAAGACATAGACATG	
miR-124a-2 3probe 31	AGCAGAAGTAGAAATCGGCTTCTCTCAG	
miR-124a-3 5probe 51	ATTTCTGCCCCGCTCGAGAGCACAGCTC	
miR-124a-3 5probe 31	GCAGTGAGGAAGGATGGCTTGGGCCATG	
miR-124a-3 3probe 51	CGATGTTGTCCACGACTCCGTACAGGCA	
miR-124a-3 3probe 31	GCTCACAGCTGTCTATGGGCAAGCTGTC	
qPCR primer		
NeuroD1-F	TCCAGGGTTATGAGATCGTCA	
NeuroD1-R	TCGCTGTATGATTTGGTCATG	
GAD2-F	GTGGAAGCTGAGTGGAGTAGAGAG	
GAD2-R	GTCTCCTGTGTCATAGGACAGGTC	
Vglut1-F	TGCCAGCATCTTGATGGGCATTTC	
Vglut1-R	CTATGAGGAACACGTACTGCCAC	
Gfap-F	GTTAAGCTAGCCCTGGACATCGAG	
Gfap-R	GATCTGGAGGTTGGAGAAAGTCTG	
Nanog-F	GAACTCTCCTCCATTCTGAACCTG	
Nanog-R	AGACCATTGCTAGTCTTCAACCAC	
Tuj1-F	GACTTGGAACCTGGAACCATGGAC	
Tuj1-R	CAGTTGTTGCCAGCACCACTCTGA	
Map2-F	GACAATGCTCACCACGTACCTGGA	
Map2-R	GATGATCTCAGCCCCGTGATCTAC	
Itgb1-F	AGGTCGATCCTGTGACCCATTGCA	
Itgb1-R	GAACAATTCCAGCAACCACGCCTG	
mVim-QPCR-51	GGAGATGCTCCAGAGAGAGGAAG	
mVim-QPCR-31	CAAGGATTCCACTTTCCGTTCAAG	
mS100b-QPCR-51	AGCACAAGCTGAAGAAGTCAGAAC	
mS100b-QPCR-31	CACCACTTCCTGCTCCTTGATTTC	
mAldh1l1-QPCR-51	TTTCTTCAACAAAGGGGAGAACTG	
mAldh1l1-QPCR-31	GTTGCCGATTTTCATCTTCCCTAC	
mTardbp-QPCR-51	GTGTGACTGTAAACTTCCCAACTC	
mTardbp-QPCR-31	TACAACGTCCAACAAACACCTTTC	
mSrebf2-QPCR-51	ATCCTACCAAGCACACTGATTGAG	
mSrebf2-QPCR-31	CAGACTCTGGGCACGATTTAAGAA	
mNupr1-QPCR-51	GAATATGATCAGTACAGCCTGGC	46
mNupr1-QPCR-31	CAGAGTTCTGGAACTTGGTCAGC	46
mKlf6-QPCR-51	ACCCGACATGGATGTGCTCCCAAT	47
mKlf6-QPCR-31	GCAGGGCTCACTCTGAAGATA	47
Rpl4-F	GATATGCCATCTGTTCTGCCCT	
Rpl4-R	CTTGCCAGCTCTCATTCTCTGA	
Construct		
mTardbp-ORF-SalI-51	GGGGTCGACATGTCTGAATATATTCGGGTAACAGAA	
mTardbp-ORF-NotI-31	GGGGCGGCCGCCTACATTCCCCAGCCAGAAGACTTAGA	
CRISPR		
mir124-1-sgRNA-F	GTCGACTAATACGACTCACTATAGGTCCATACAATTAAGGCACG	
mir124-2-sgRNA-F	GTCGACTAATACGACTCACTATAGTGTCATACAATTAAGGCACG	
mir124-3-sgRNA-F	GTCGACTAATACGACTCACTATAGGTCTATACAATTAAGGCACG	
sgRNA-R	GGATCCAAAAGCACCGACTCGGTGCC	
Genotyping		
miR-124a-2-tail-F	TAGGTGCGCTGTAAATGGCATG	
miR-124a-2-tail-R	TGAATCAATGCGAGGGGTCCT	
miR-124a-3-tail-F	GTGATACTCATGACATCCCTTCGT	
miR-124a-3-tail-R	TCTGCGTGTTCACAGCGGACCTTGAT	
pPNT-lox-neo-F	ATATGATCGGAATTGGTCTCCCG	