Neuron D-09-00916 revision 2 (version 3.0)

Dicer1 and miR-219 Are Required for Normal Oligodendrocyte Differentiation and Myelination, J. C. Dugas et al.

INVENTORY OF SUPPLEMENTAL INFORMATION

Supplemental Data: non-printable items
Movie M1 and M2. Movies showing shivering phenotype of mutant (M2) vs. littermate control (M1) mice, related to Figure 1.

Supplemental Data: figures
Figure S1. Quantification of behavioral phenotypes illustrated in M1 and M2, related to Figure 1.
Figure S2. Additional visualization and quantification of in vivo developmental myelination phenotypes in mutant / wt mice, related to Figure 1.
Figure S3. Additional visualization and quantification of in vivo developmental OL differentiation phenotypes in mutant / wt mice, related to Figure 2.
Figure S4. Quantification of Dicer1 expression in mutant vs. wt OLs in vivo and in vitro, and in OPCs vs. OLs in vitro, related to Figure 3.
Figure S5. Additional *in situ* images associated with Figure 4.
Figure S6. Additional staining images (CNP and MOG expression) and quantification of data associated with Figure 5.
Figure S7. Additional analysis of miRNAs identified in Figure 4 and analyzed in Figure 5 and 6 (analysis of genes targeted by selected miRNAs), and verification of expression of *FoxJ3* and *ZFP238* expression in OPCs / OLs, related to Figure 6.
Figure S8. Additional data associated miR-219 repression of target genes, and investigation of the biological role of miR-219 targeted genes *FoxJ3* and *ZFP238*, related to Figure 7.

Supplemental Data: tables
Table S1. Table showing top identified candidate miRNAs induced during OL differentiation, related to Figure 4.
Table S2-S4. Tables showing potential targets of miR-219 ranked by context score (S1), level of repression during OL differentiation (S2), or expression level in OPCs (S3), related to Figure 6 and Figure S7.

Supplemental Experimental Procedures

Neuron D-09-00916 revision 2

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SUPPLEMENTAL MATERIAL

Movies M1 and M2. Mice lacking functional Dicer1 expression in OPCs and OLs exhibit a shivering phenotype. A P16 mouse lacking Dicer1 function in its OPCs and OLs, *Olig2*$^{Cre/+}$*;Dicer1*$^{f/f}$ (M2), exhibits a shivering phenotype, reminiscent of previously reported CNS myelination defects. A littermate control *Olig2*$^{Cre/+}$*;Dicer1*$^{f/+}$ mouse (M1) does not show this phenotype.

Figure S1

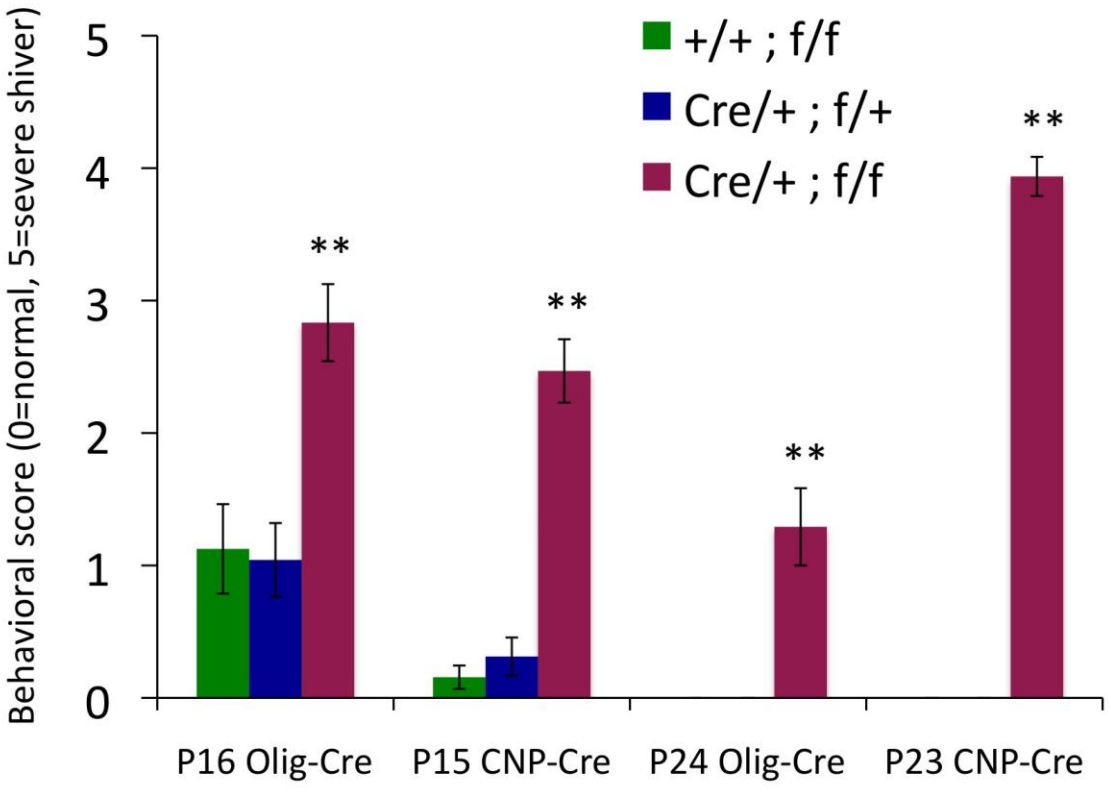


Figure S1. Mice lacking functional Dicer1 in OPCs and OLs exhibit a shivering phenotype, related to Figure 1. Quantification of the severity of the "shivering" phenotype in P15-16 and P23-24 mutant *Olig2*$^{Cre/+}$*;Dicer1*$^{f/f}$ and *CNP*$^{Cre/+}$*;Dicer1*$^{f/f}$ mice (red bars) compared to control littermate *Olig2*$^{+/+}$*;Dicer1*$^{f/f}$ and *CNP*$^{+/+}$*;Dicer1*$^{f/f}$ mice (green bars) and *Olig2*$^{Cre/+}$*;Dicer1*$^{f/+}$ and *CNP*$^{Cre/+}$*;Dicer1*$^{f/+}$ mice (blue bars). Scores were generated by four different blinded observers on an arbitrary 0-5 (normal – severe defect) scale. Error bars ±S.E.M., n=3-4 mice / genotype. ** $p < 0.001$, post-hoc all pairwise SNK tests, mutant compared to either control condition.

Figure S2

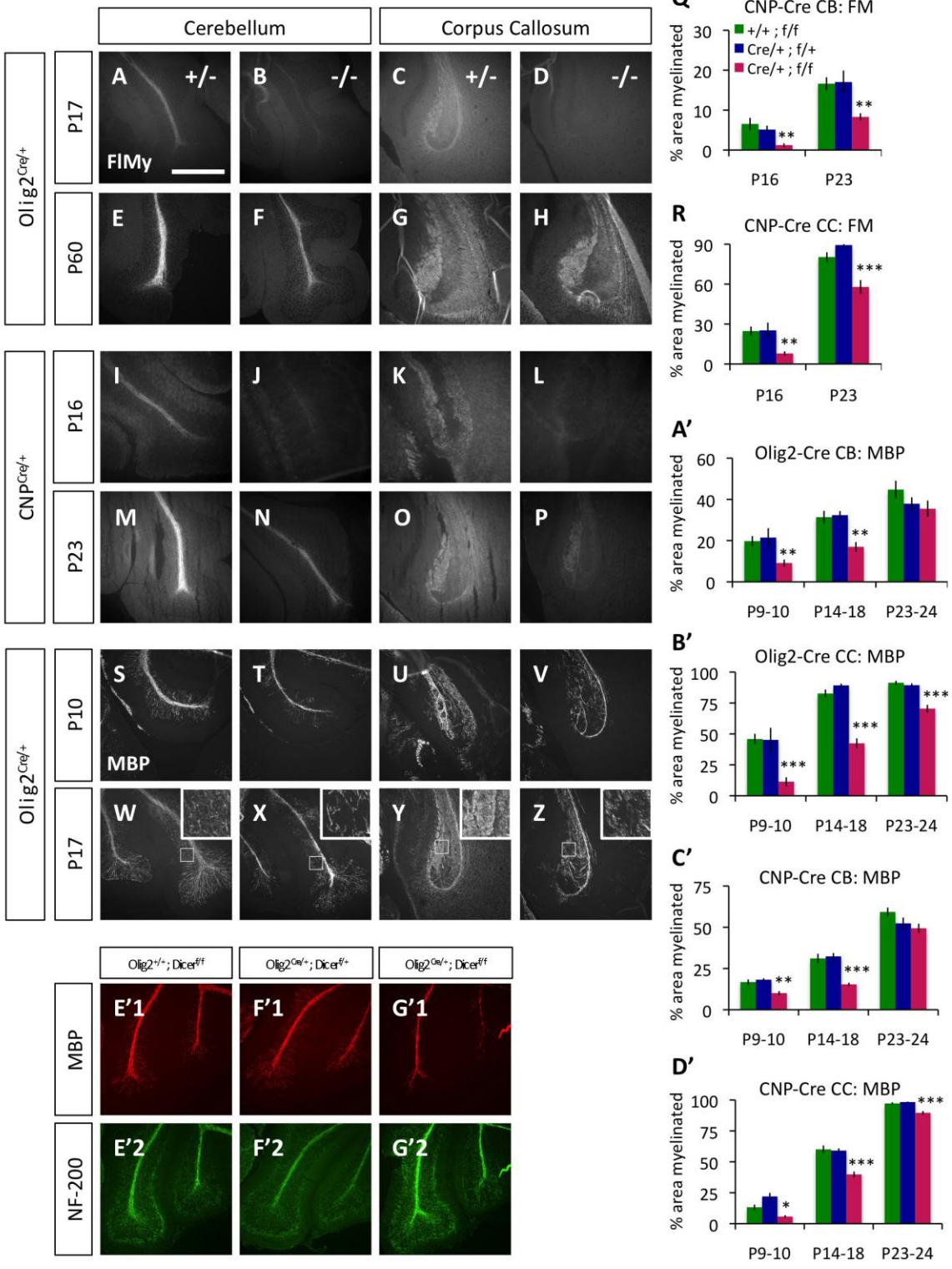

Cerebellum
Corpus Callosum
Olig2Cre/+
CNPCre/+
P17
P60
P16
P23
P10
A +/-
B -/-
C +/-
D -/-
FIMy
E
F
G
H
I
J
K
L
M
N
O
P
S
T
U
V
MBP
W
X
Y
Z
Olig2+/+; Dicerf/f
Olig2Cre/+; Dicerf/+
Olig2Cre/+; Dicerf/f
MBP
NF-200
E'1
F'1
G'1
E'2
F'2
G'2
Q
CNP-Cre CB: FM
+/+ ; f/f
Cre/+ ; f/+
Cre/+ ; f/f
% area myelinated
P16
P23
R
CNP-Cre CC: FM
A'
Olig2-Cre CB: MBP
P9-10
P14-18
P23-24
B'
Olig2-Cre CC: MBP
C'
CNP-Cre CB: MBP
D'
CNP-Cre CC: MBP

Figure S2. Disruption of *Dicer1* in *Olig2$^{Cre/+}$;Dicer1$^{f/f}$* and *CNP$^{Cre/+}$;Dicer1$^{f/f}$* mice specifically delays compact myelin formation in the CNS, related to Fig. 1. A-H. Images of FlMy staining in the cerebellar arms (A,B,E,F) and splenium regions of the corpus callosum (C,D,G,H) from similar depth sagital brain sections of P17 (A-D) and P60 (E-H) mutant *Olig2$^{Cre/+}$;Dicer1$^{f/f}$* (B,F,D,H) and littermate control *Olig2$^{Cre/+}$;Dicer1$^{f/+}$* (A,E,C,G) mice. **I-P.** Images of FlMy staining in the cerebellar arms (I,J,M,N) and splenium regions of the corpus callosum (K,L,O,P) from similar depth sagital brain sections of P16 (I-L) and P23 (M-P) mutant *CNP$^{Cre/+}$;Dicer1$^{f/f}$* (J,N,L,P) and littermate control *CNP$^{Cre/+}$;Dicer1$^{f/+}$* (I,M,K,O) mice. **Q-R.** Quantification of the percentage area myelinated, as determined by FlMy staining, in the cerebellar arms (Q) and corpora callosa (R) of mutant *CNP$^{Cre/+}$;Dicer1$^{f/f}$* and littermate control mice at P14-16 and P22-23. **S-Z.** Images of MBP expression in the cerebellar arms (S,T,W,X) and splenium regions of the corpus callosum (U,V,Y,Z) from similar depth sagital brain sections of P10 (S-V) and P17 (W-Z) mutant *Olig2$^{Cre/+}$;Dicer1$^{f/f}$* (T,X,V,Z) and littermate control *Olig2$^{Cre/+}$;Dicer1$^{f/+}$* (S,W,U,Y) mice. Boxed regions in W-Z are shown at higher magnification in the upper right corner of each panel. **A'-D'.** Quantification of the percentage area myelinated, as determined by MBP staining, in the cerebellar arms (A',C') and corpora callosa (B',D') of control *Olig2$^{+/+}$;Dicer1$^{f/f}$*, control *Olig2$^{Cre/+}$;Dicer1$^{f/+}$*, and mutant *Olig2$^{Cre/+}$;Dicer1$^{f/f}$* mice (A'-B') or control *CNP$^{+/+}$;Dicer1$^{f/f}$*, control *CNP$^{Cre/+}$;Dicer1$^{f/+}$*, and mutant *CNP$^{Cre/+}$;Dicer1$^{f/f}$* mice (C'-D') at P9-10, P14-18, and P23-24. **E'-G'** Immunostaining of sagital sections of the cerebellar arms of mutant P10 *Olig2$^{Cre/+}$;Dicer1$^{f/f}$* mice (E') and control genotype littermates (F'-G') for myelin (MBP: E'1-G'1) and axons (NF-200: E'2-G'2). Scale bar = 500μm all images. All graphs error bars ±S.E.M., n=3-5 animals / condition. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ post-hoc all pairwise SNK tests, mutant compared to either control condition.

Figure S3

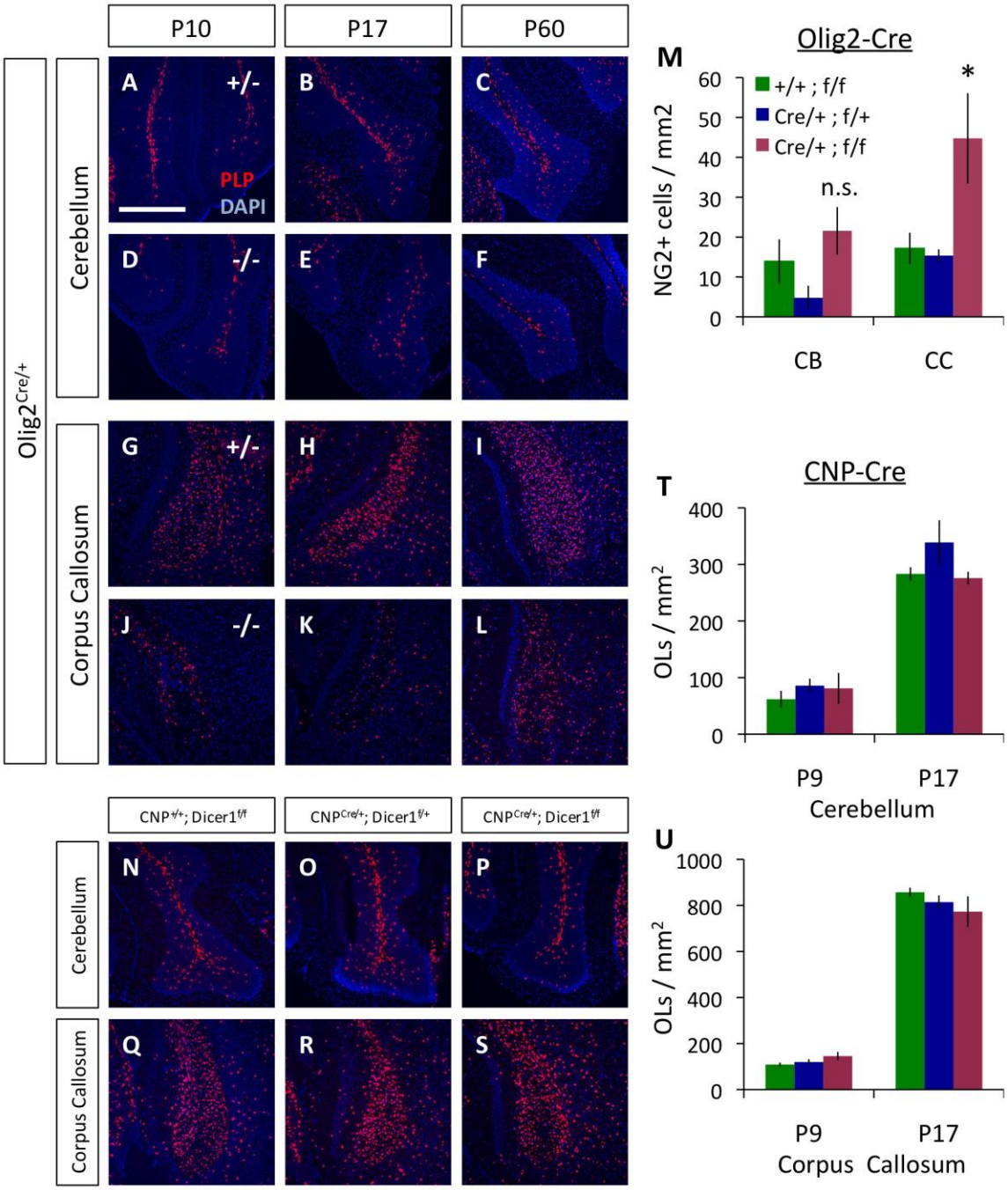

P10
P17
P60
Olig2Cre/+
Cerebellum
Corpus Callosum
A
+/-
B
C
PLP
DAPI
D
-/-
E
F
G
+/-
H
I
J
-/-
K
L
M
Olig2-Cre
+/+ ; f/f
Cre/+ ; f/+
Cre/+ ; f/f
n.s.
*
NG2+ cells / mm2
60
50
40
30
20
10
0
CB
CC
T
CNP-Cre
OLs / mm2
400
300
200
100
0
P9
P17
Cerebellum
CNP+/+; Dicer1f/f
CNPCre/+; Dicer1f/+
CNPCre/+; Dicer1f/f
N
O
P
Q
R
S
U
1000
800
600
400
200
0
P9
P17
Corpus Callosum

Figure S3. Mature OL numbers are reduced in mice lacking Dicer1 function in OPCs and OLs, but not in mice lacking Dicer1 function only in OLs, related to Fig. 2. A-L. *In situs* for PLP expression (red) to visualize mature OLs in the cerebellar arms (A-F) and splenium regions of the corpus callosum (G-L) from similar depth sagital brain sections of P10 (A,D,G,J), P17 (B,E,H,K), and P60 (C,F,I,L) littermate control *Olig2*$^{Cre/+}$*;Dicer1*$^{f/+}$ (A-C, G-I) and mutant *Olig2*$^{Cre/+}$*;Dicer1*$^{f/f}$ (D-F, J-L) mice. **M.** Quantification of immature OPCs (NG2^{+} cells) / mm^{2} in the cerebellar arms and corpora callosa of mutant *Olig2*$^{Cre/+}$*;Dicer1*$^{f/f}$ and littermate control mice at P24. Error bars ±S.E.M., n=3-4 animals / condition. * $p < 0.05$, n.s. = not significant post-hoc Holm-Sidak test, mutant compared to either control condition. No significant changes in NG2^{+} cell numbers were observed in P10 or P17 mutants relative to littermate controls. **N-S.** *In situs* for PLP expression to visualize mature OLs in the cerebellar arms (N-P) and splenium regions of the corpus callosum (Q-S) from similar depth sagital brain sections of a P17 littermate control *CNP*$^{+/+}$*;Dicer1*$^{f/f}$ (N,Q) and *CNP*$^{Cre/+}$*;Dicer1*$^{f/+}$ (O,R) mouse and a mutant *CNP*$^{Cre/+}$*;Dicer1*$^{f/f}$ (P,S) mouse. **T-U.** Quantification of mature OLs / mm^{2} in the cerebellar arms (T) and corpora callosa (U) of mutant *CNP*$^{Cre/+}$*;Dicer1*$^{f/f}$ and littermate control mice at P9 and P17. No significant differences were detected between mutant and control genotypes. All images scale bar = 500µm.

Figure S4

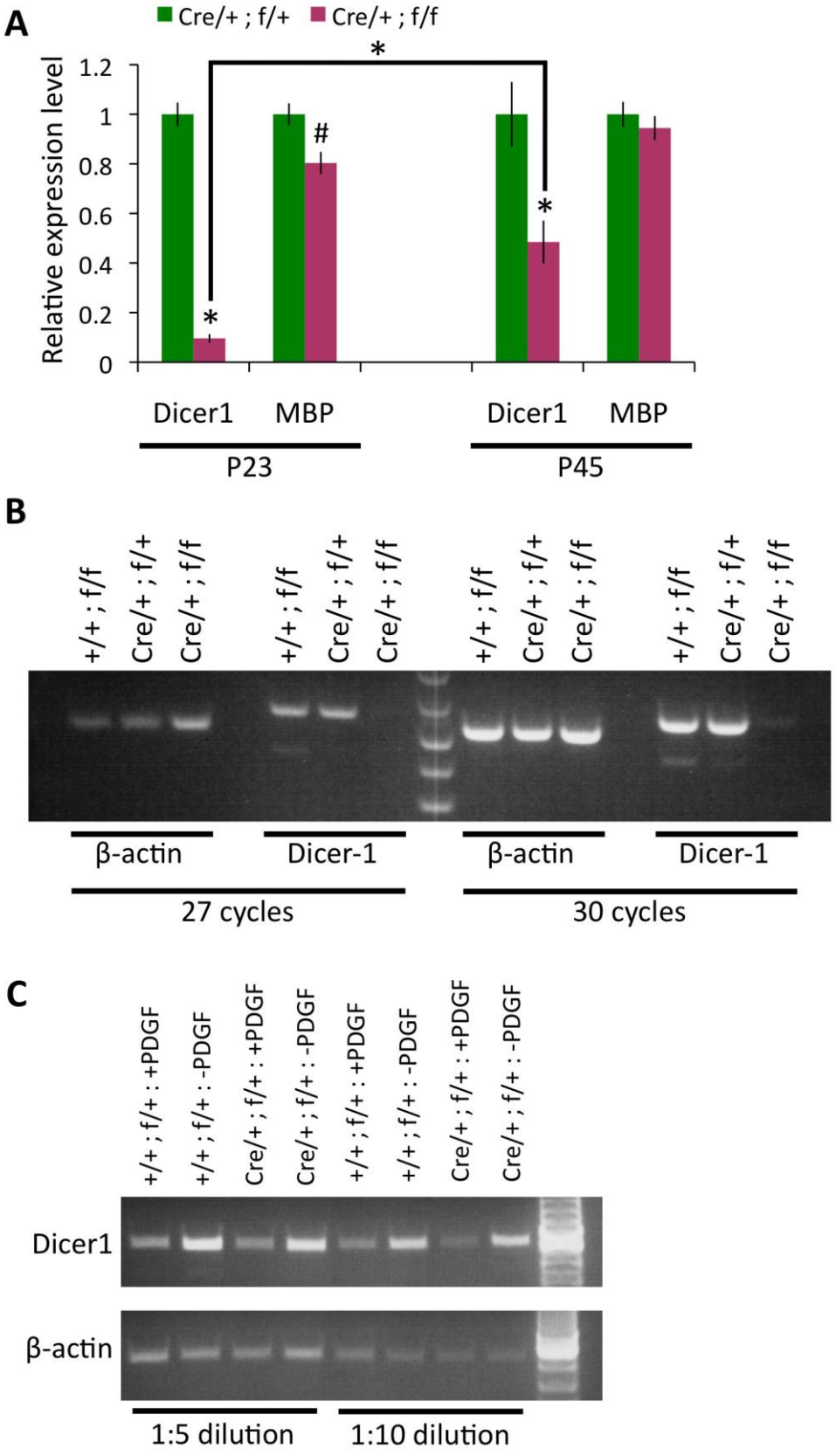

A
Cre/+ ; f/+
Cre/+ ; f/f
Relative expression level
1.2
1
0.8
0.6
0.4
0.2
0
*
#
*
*
Dicer1
MBP
Dicer1
MBP
P23
P45
B
+/+ ; f/f
Cre/+ ; f/+
Cre/+ ; f/f
β-actin
Dicer-1
β-actin
Dicer-1
27 cycles
30 cycles
C
+/+ ; f/+ : +PDGF
+/+ ; f/+ : -PDGF
Cre/+ ; f/+ : +PDGF
Cre/+ ; f/+ : -PDGF
Dicer1
β-actin
1:5 dilution
1:10 dilution

Figure S4. *Dicer1* expression in mutant and control genotype OLs *in vivo* and *in vitro*, related to Fig. 3. A. qRT-PCR to detect Dicer1 and MBP expression in *Olig2*$^{Cre/+}$*;Dicer1*$^{f/+}$ (Cre/+;f/+) and *Olig2*$^{Cre/+}$*;Dicer1*$^{f/f}$ (Cre/+;f/f) OL samples acutely purified from P23 or P45 littermates. Error bars ±S.E.M.; two (P45) or four (P23) biological replicate OL samples were obtained for each genotype, with 3x independent qRT-PCR runs performed per replicate. * $p < 0.001$ post-hoc all pairwise SNK tests, mutant compared to paired control or P23 vs. P45 mutant OL Dicer1 levels. # $p < 0.01$ T-test mutant vs. control P23 MBP expression ($p > 0.05$ in all pairwise SNK tests). **B.** Semi-quantitative RT-PCR of *CNP*$^{+/+}$*;Dicer1*$^{f/f}$ (+/+;f/f), *CNP*$^{Cre/+}$*;Dicer1*$^{f/+}$ (Cre/+;f/+), and *CNP*$^{Cre/+}$*;Dicer1*$^{f/f}$ (Cre/+;f/f) OPCs cultured for 4 DIV in -PDGF-T3 media. RT reactions were performed with 400ng RNA from each sample, and RT reaction products were diluted 1:3 before use in PCR reactions for Dicer1 or 1:30 for β-actin (27 and 30 cycles each). **C.** Semi-quantitative RT-PCR of *CNP*$^{+/+}$*;Dicer1*$^{f/+}$ (+/+;f/+) and *CNP*$^{Cre/+}$*;Dicer1*$^{f/+}$ (Cre/+;f/+) OPCs cultured for 3 DIV in +PDGF-T3 (+PDGF) or -PDGF-T3 (-PDGF) media. RT reactions were performed with 400ng RNA from each sample, and RT reaction products were diluted 1:5 or 1:10 before use in PCR reactions for Dicer1 (32 cycles) or β-actin (24 cycles).

Figure S5

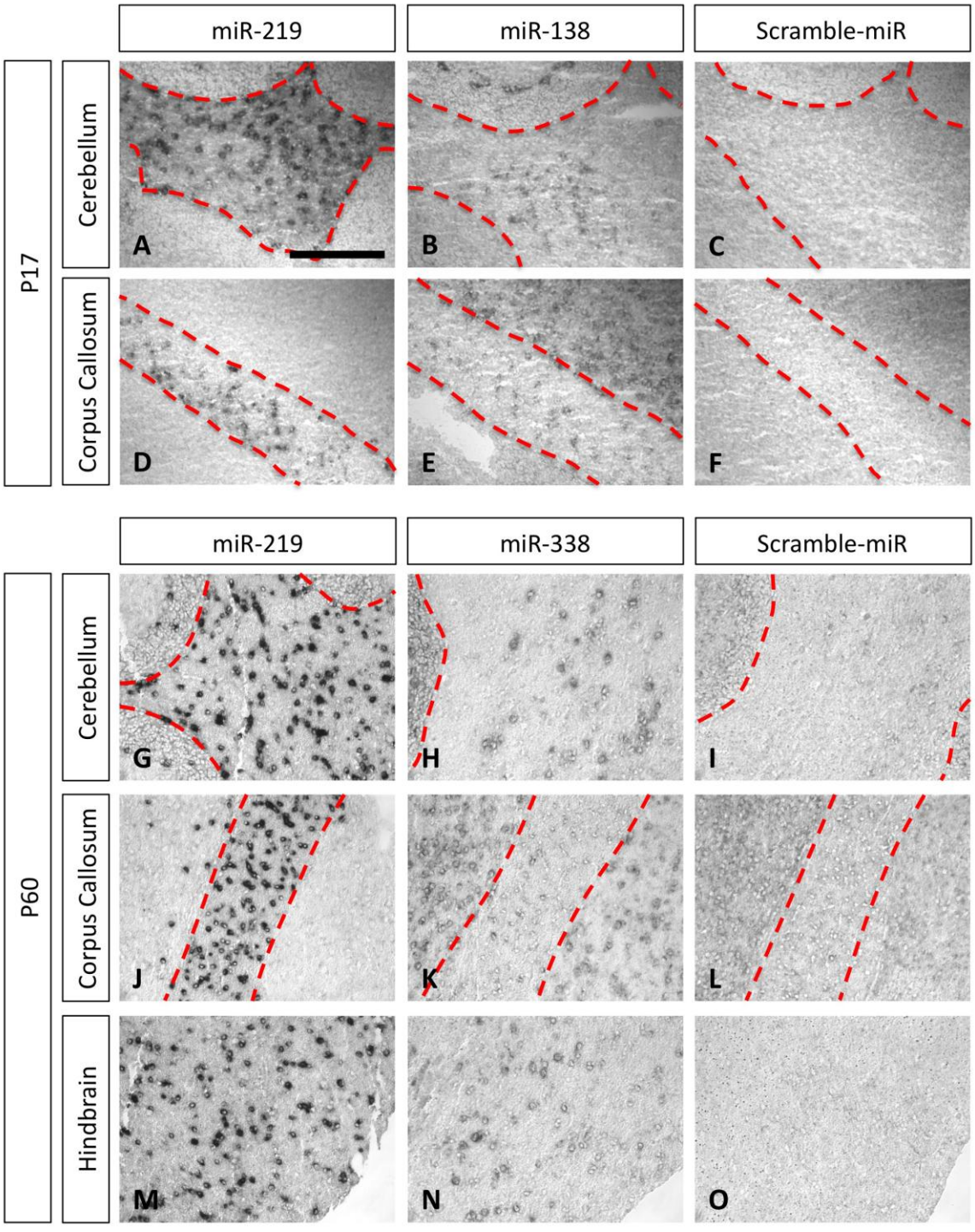
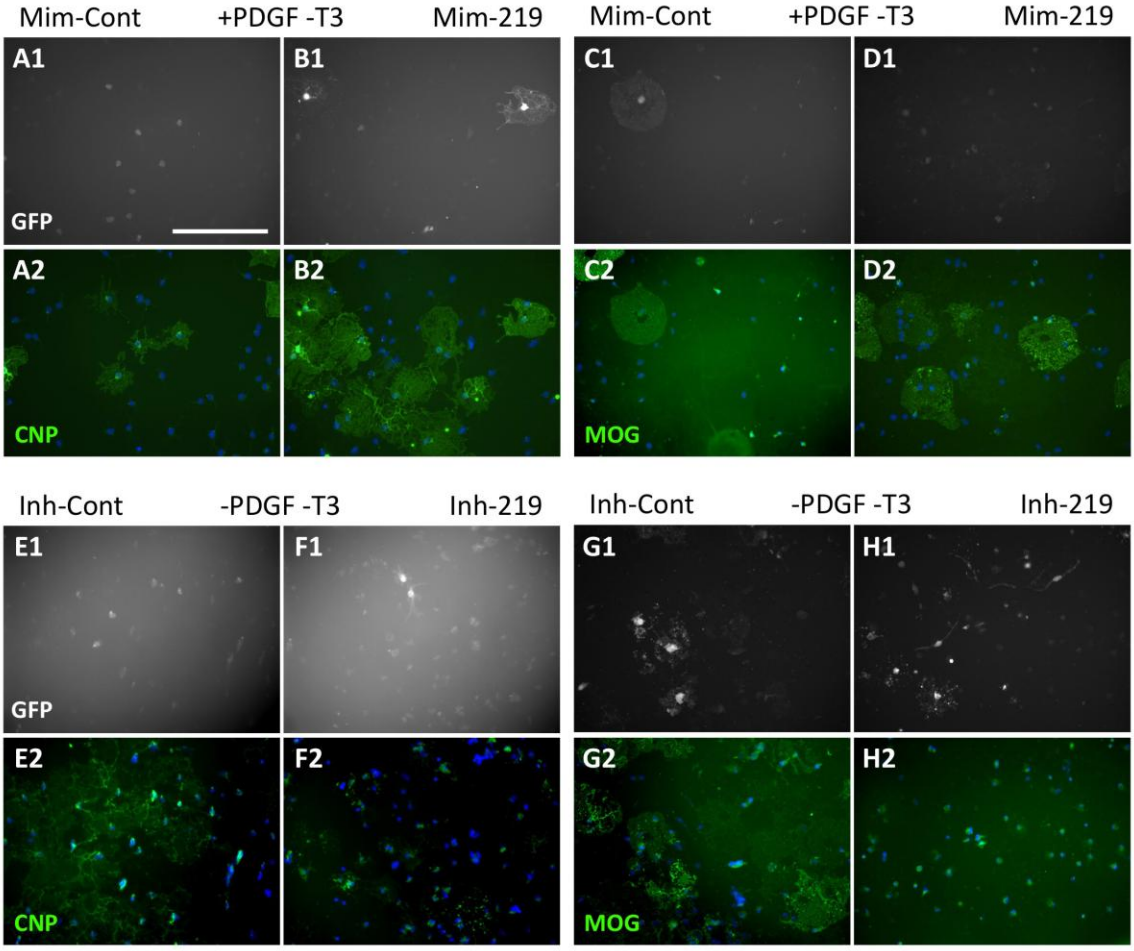

miR-219
miR-138
Scramble-miR
P17
Cerebellum
Corpus Callosum
A
B
C
D
E
F
miR-219
miR-338
Scramble-miR
P60
Cerebellum
Corpus Callosum
Hindbrain
G
H
I
J
K
L
M
N
O

Figure S5. miR-219, miR-138, and miR-338 are expressed in CNS white matter, related to Fig. 4. *In situ* hybridizations to visualize miR-219 (A, D, G, J, M), miR-138 (B, E), or miR-338 (H, K, N) expression in P17 (A-F) or P60 (G-O) central cerebellar region (A-C, G-I), corpus callosum (D-F, J-L), or hindbrain (M-O) relative to Scramble-miR negative control (C, F, I, L, O). Scale bar = 200 μm.

Figure S6


Mim-Cont
+PDGF -T3
Mim-219
Mim-Cont
+PDGF -T3
Mim-219
A1
B1
C1
D1
GFP
A2
B2
C2
D2
CNP
MOG
Inh-Cont
-PDGF -T3
Inh-219
Inh-Cont
-PDGF -T3
Inh-219
E1
F1
G1
H1
GFP
E2
F2
G2
H2
CNP
MOG

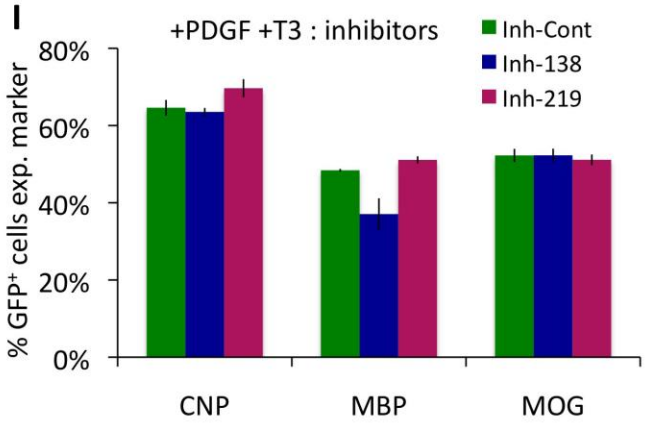
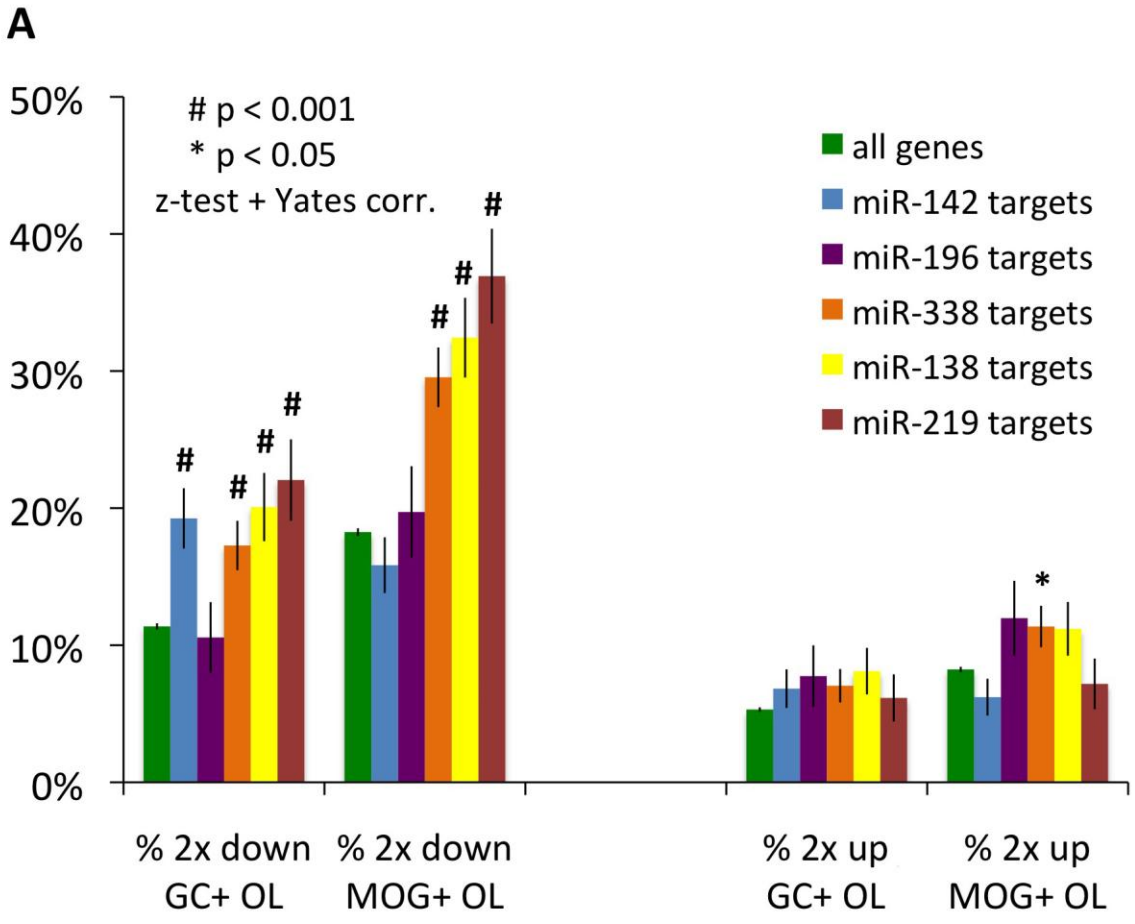

I
+PDGF +T3 : inhibitors
Inh-Cont
Inh-138
Inh-219
% GFP+ cells exp. marker
80%
60%
40%
20%
0%
CNP
MBP
MOG

Figure S6. miR-219 regulates normal mitogen-withdrawal induced, but not T3 induced, OL differentiation, related to Fig. 5. A-H. Transfected OPCs, identified by GFP expression (white, A1-H1), stained for myelin gene expression (green, CNP: A2-B2, E2-F2; MOG: C2-D2, G2-H2); nuclei marked by DAPI staining (blue, A2-H2). Scale bar = 200 µm. **A-D.** OPCs transfected with control mimic (A,C) or miR-219 mimic (B,D) cultured for 7 DIV in +PDGF-T3 media. **E-H.** OPCs transfected with control inhibitor (E,G) or miR-219 inhibitor (F,H) cultured for 3-4 DIV in -PDGF-T3 media. **I.** Percentages of transfected, GFP^+ cells expressing the indicated markers (CNP, MBP, or MOG). OPCs were transfected with control, miR-138, and miR-219 inhibitors and cultured for 7 DIV in +PDGF+T3 media. Error bars ±S.E.M., n=6. No significant differences detected post-hoc Holm-Sidak test vs. control.

Figure S7


A
50%
40%
30%
20%
10%
0%
# p < 0.001
* p < 0.05
z-test + Yates corr.
all genes
miR-142 targets
miR-196 targets
miR-338 targets
miR-138 targets
miR-219 targets
% 2x down GC+ OL
% 2x down MOG+ OL
% 2x up GC+ OL
% 2x up MOG+ OL

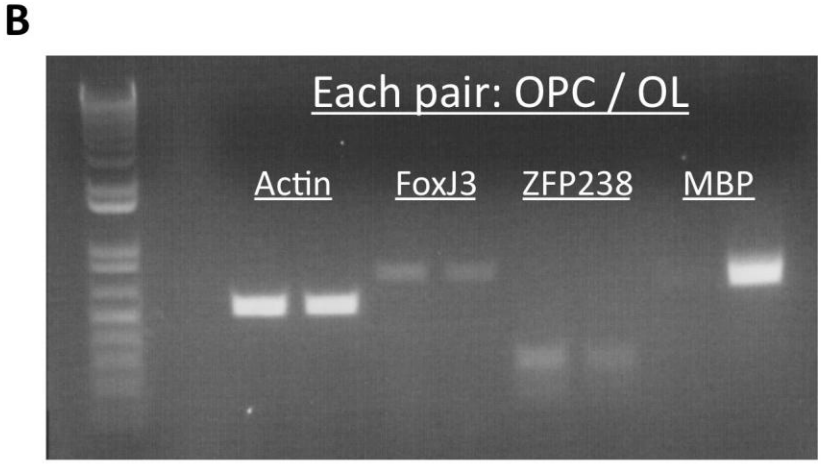

B
Each pair: OPC / OL
Actin
FoxJ3
ZFP238
MBP

Figure S7. miR-219, miR-138, and miR-338 preferentially target genes repressed during OL differentiation, related to Fig. 6. A. Lists of common predicted mammalian target genes for miR-219, miR-138, miR-338, and also miR-142 and miR-196 (enriched in hematopoetic cells and kidney, respectively (Landgraf et al., 2007)), were obtained from TargetScan 5.1 (http://www.targetscan.org/). To reduce the likelihood of including "false" targets, we only included predicted targets that were present on at least two mammalian lists (human, mouse, or rat). The percentages of these genes that are induced or repressed during OL differentiation were determined by comparing these lists to the sets of all OL-lineage expressed genes that are increased > 2-fold in immature MOG^- GC^+ OLs relative to OPCs (2x up GC+ OL) or mature MOG^+ OLs relative to OPCs (2x up MOG+ OL), or decreased > 2-fold in immature MOG^- GC^+ OLs or mature MOG^+ OLs relative to OPCs (2x down GC+ / MOG+ OL) (Cahoy et al., 2008). For instance, for miR-219, % 2x down MOG+ OL = 72 miR-219 predicted targets that are repressed ≥ 2-fold in MOG^+ OLs relative to OPCs / 195 total miR-219 predicted targets. "all genes" = the total percentage of unique OL-lineage expressed genes that are induced or repressed during OL differentiation. For instance, % 2x down MOG+ OL = 3500 unique OL-lineage expressed genes that are repressed ≥ 2-fold in MOG^+ OLs relative to OPCs / 19177 total unique, characterized genes. "OL-lineage expressed" is defined as an expression level ≥ 250 in the OPC, GC+ OL, or MOG+ OL sample. Error bars ± standard error of the proportion. * $p < 0.05$, # $p < 0.001$ z-test with Yates correction, comparing percentage repressed/induced predicted miRNA targeted genes vs. total percentage repressed/induced genes ("all genes"). **B.** Semi-quantitative RT-PCR confirms expression of *FoxJ3* and *ZFP238* in OPCs and approximately 2-fold reduction of expression in OLs. Purified OPCs were cultured for 4 DIV in +PDGF-T3 media (OPC) or -PDGF-T3 media (OL). RT reactions were performed with 400ng RNA from each sample, and RT reaction products were diluted 1:20 before use in PCR reactions for Actin (27 cycles), and 1:2 for *FoxJ3* (24 cycles), *ZFP238* (30 cycles) or *MBP* (27 cycles) reactions.

Figure S8

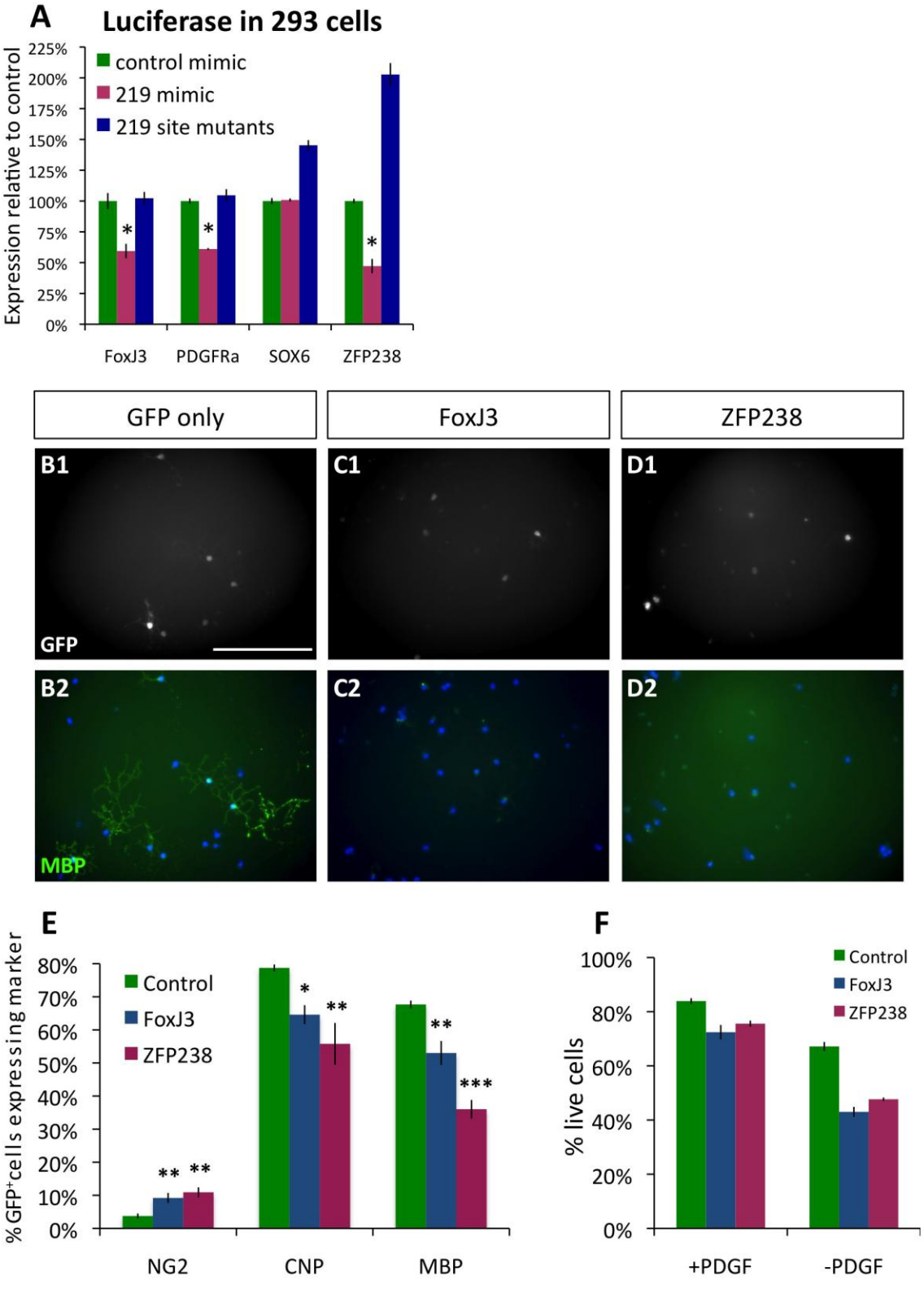

A
Luciferase in 293 cells
Expression relative to control
225%
200%
175%
150%
125%
100%
75%
50%
25%
0%
control mimic
219 mimic
219 site mutants
*
FoxJ3
PDGFRa
SOX6
ZFP238
GFP only
FoxJ3
ZFP238
B1
C1
D1
GFP
B2
C2
D2
MBP
E
% GFP+ cells expressing marker
80%
70%
60%
50%
40%
30%
20%
10%
0%
Control
FoxJ3
ZFP238
**
*
***
NG2
CNP
MBP
F
% live cells
100%
80%
60%
40%
20%
0%
Control
FoxJ3
ZFP238
+PDGF
-PDGF

Figure S8. miR-219 represses the expression of *PDGFRα, Sox6, FoxJ3*, and *ZFP238*; FoxJ3 and ZFP238 repress OL differentiation, related to Fig. 7. A. Levels of normalized luciferase activity detected at 1 DIV in 293 co-transfected with indicated luciferase reporter constructs and either control (green bars) or miR-219 (red bars) mimic, blue bars represent co-transfections of miR-219 mimic with reporter constructs containing mutated 219 binding sites; data displayed relative to control mimic co-transfection luciferase activity levels. Error bars ±S.E.M., n = 8-12. * $p < 0.001$ T-test control vs. miR-219 mimic transfections (T-tests 219 mutants vs. control not done). **B-D.** OPCs transfected with only the GFP-expression plasmid (B), FoxJ3 expression plasmid (C), or ZFP238 expression plasmid (D) are identified by GFP expression (white, B1-D1) and stained for MBP expression (green, B2-D2). Nuclei marked by DAPI staining (blue, B2-D2). Transfected cells cultured 3 DIV in -PDGF-T3 media. Scale bar = 200 μm. **E.** Percentages of healthy transfected, GFP^+ cells expressing the indicated markers (OPCs: NG2; OLs: CNP or MBP). Error bars ±S.E.M., n=5-6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ post-hoc Holm-Sidak test vs. control. **F.** Survival assay for OPCs transfected with GFP, FoxJ3, or ZFP238 expression plasmids, cultured 2 DIV in +PDGF-T3 or -PDGF-T3 media (statistics not done).

Table S1. Top candidate miRNAs induced during OL differentiation

Name	log2 FC	fold activation	Ave Expr	P Value	B Stat
hsa/mmu/dre-mir-219	4.3	19.9	7.5	5.4E-12	15.0
dre-mir-138	2.9	7.3	6.4	2.9E-09	10.8
hsa/mmu/dre-mir-338	3.1	8.6	6.4	1.3E-07	7.7
hsa/mmu-miR-181c	1.4	2.7	6.9	1.2E-06	5.8
dre-mir-26b	1.0	2.0	7.6	2.2E-06	5.2
hsa/mmu/dre-mir-181a	1.0	2.0	8.6	4.9E-06	4.3
cand4right	0.7	1.6	9.7	8.8E-06	3.8
hsa/mmu/dre-mir-103	1.1	2.1	8.1	1.2E-05	3.5
hsa/mmu-mir-181d	1.2	2.3	7.6	2.3E-05	2.8

Table S1. Top candidate miRNAs induced during OL differentiation, related to Fig. 4. The nine most statistically significant differentially expressed miRNAs calculated from the microarrays are listed, based on the B statistic (B Stat.) a measure of the probability that the observed differences in expression are real. Log_2 FC represents the log_2 fold change in expression between OPCs and OLs. Avg. Expr. is the average intensity observed for each miRNA.

Table S2. Top 25 miR-219 target genes: context score

Gene Symbol	Gene Title	Avg context	OPC	GC+ OL	MOG+ OL	MOG+ / OPC
Zfp238	zinc finger protein 238	-0.930	2997	1824	1360	0.454
Glt8d3	glycosyltransferase 8 domain containing 3	-0.707	610	755	769	1.261
Foxj3	forkhead box J3	-0.673	2755	1384	781	0.283
Cd2ap	CD2-associated protein	-0.593	970	824	801	0.826
Ubr1	ubiquitin protein ligase E3 component n-recognin 1	-0.547	1907	1867	2282	1.197
Sorcs1	VPS10 domain receptor protein SORCS 1	-0.503	1018	748	817	0.803
Ash1l	ash1 (absent, small, or homeotic)-like (Drosophila)	-0.500	1088	997	945	0.869
Snrk	SNF related kinase	-0.497	2191	699	388	0.177
Rims1	regulating synaptic membrane exocytosis 1	-0.480	973	705	838	0.861
Cd164	CD164 antigen	-0.467	5830	4152	4280	0.734
Nr2c2	nuclear receptor subfamily 2, group C, member 2	-0.460	4217	3074	2175	0.516
Mef2d	myocyte enhancer factor 2D	-0.457	1032	978	638	0.618
Elovl7	ELOVL family member 7, elongation of long chain fatty acids (yeast)	-0.447	358	8547	13437	37.527
Cc2d1a	coiled-coil and C2 domain containing 1A	-0.430	273	103	123	0.451
Col9a1	procollagen, type IX, alpha 1	-0.427	392	1405	942	2.404
Sp4	trans-acting transcription factor 4	-0.427	366	287	243	0.663
Fzd4	frizzled homolog 4 (Drosophila)	-0.420	307	120	33	0.106
Reck	reversion-inducing-cysteine-rich protein with kazal motifs	-0.417	893	622	401	0.450
Mier3	mesoderm induction early response 1, family member 3	-0.417	760	513	559	0.735
Pdgfra	platelet derived growth factor receptor, alpha polypeptide	-0.397	24131	13069	36	0.001
Egr3	early growth response 3	-0.393	307	63	85	0.277
Cugbp2	CUG triplet repeat, RNA binding protein 2	-0.373	8527	2792	790	0.093
D630045J12Rik	RIKEN cDNA D630045J12 gene	-0.373	1899	4172	1368	0.720
Dnajc6	DnaJ (Hsp40) homolog, subfamily C, member 6	-0.367	189	616	3892	20.564
Dazap1	DAZ associated protein 1	-0.363	498	801	850	1.707

Table S2. Top 25 miR-219 target genes: context score, related to Fig. 6. Top miR-219 predicted target genes, ranked by context score, as determined from TargetScan 5.1 (http://www.targetscan.org/). OPC, GC+ OL, and MOG+ OL expression levels obtained from Cahoy et al. (2008). MOG+ / OPC = MOG+ OL expression / OPC expression. Genes selected for further study are highlighted.

Table S3. Top 25 miR-219 target genes: OL repressed genes

Gene Symbol	Gene Title	Avg context	OPC	GC+ OL	MOG+ OL	MOG+ / OPC
Pdgfra	platelet derived growth factor receptor, alpha polypeptide	-0.397	24131	13069	36	0.001
Ppargc1a	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	-0.167	588	132	10	0.016
Etv5	ets variant gene 5	-0.193	3087	509	66	0.021
Ddah1	dimethylarginine dimethylaminohydrolase 1	-0.293	7386	1877	159	0.021
Eya1	eyes absent 1 homolog (Drosophila)	-0.220	2081	465	50	0.024
1110067D 22Rik	RIKEN cDNA 1110067D22 gene	-0.243	6490	1516	223	0.034
Fbxo41	F-box protein 41	-0.193	499	145	19	0.038
Nol4	nucleolar protein 4	-0.250	775	199	30	0.039
Sox6	SRY-box containing gene 6	-0.243	4976	2260	204	0.041
1700022C 02Rik	RIKEN cDNA 1700022C02 gene	-0.153	344	56	16	0.048
Lmo3	LIM domain only 3	-0.200	835	224	41	0.049
Fchsd2	FCH and double SH3 domains 2	-0.057	8415	4082	418	0.050
Lpp	LIM domain containing preferred translocation partner in lipoma	-0.353	472	139	25	0.053
AU041783	expressed sequence AU041783	-0.183	2518	1446	150	0.059
Kcnh8	potassium voltage-gated channel, subfamily H (eag-related), member 8	-0.330	304	112	19	0.061
Glce	glucuronyl C5-epimerase	-0.140	1576	527	105	0.067
Wee1	wee 1 homolog (S. pombe)	-0.173	508	118	36	0.071
Rbms1	RNA binding motif, single stranded interacting protein 1	-0.117	554	227	41	0.074
Flrt3	fibronectin leucine rich transmembrane protein 3	-0.080	1614	531	129	0.080
Gm96	gene model 96, (NCBI)	-0.220	380	178	35	0.092
Cugbp2	CUG triplet repeat, RNA binding protein 2	-0.373	8527	2792	790	0.093
Slc39a10	solute carrier family 39 (zinc transporter), member 10	-0.207	6045	3171	563	0.093
Tanc2	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 2	-0.170	3326	1142	331	0.100
Robo1	roundabout homolog 1 (Drosophila)	-0.140	1182	407	119	0.101
Fzd4	frizzled homolog 4 (Drosophila)	-0.420	307	120	33	0.106

Table S3. Top 25 miR-219 target genes: OPC expression level, related to Fig. 6. Top miR-219 predicted target genes, ranked by OPC expression level. Genes selected for further study are highlighted.

Table S4. Top 25 miR-219 target genes: OPC expression level

Gene Symbol	Gene Title	Avg context	OPC	GC+ OL	MOG+ OL	MOG+ / OPC
Pdgfra	platelet derived growth factor receptor, alpha polypeptide	-0.397	24131	13069	36	0.001
2310047A01Rik	RIKEN cDNA 2310047A01 gene	0.007	11102	3117	3028	0.273
Scarb2	scavenger receptor class B, member 2	-0.237	10833	19812	19956	1.842
Spnb2	spectrin beta 2	-0.110	8605	12072	14411	1.675
Cugbp2	CUG triplet repeat, RNA binding protein 2	-0.373	8527	2792	790	0.093
Fchsd2	FCH and double SH3 domains 2	-0.057	8415	4082	418	0.050
Ddah1	dimethylarginine dimethylaminohydrolase 1	-0.293	7386	1877	159	0.021
Nav1	neuron navigator 1	-0.143	6511	6700	5317	0.817
1110067D22Rik	RIKEN cDNA 1110067D22 gene	-0.243	6490	1516	223	0.034
Abi2	abl-interactor 2	-0.093	6345	5285	5720	0.901
Laptm4a	lysosomal-associated protein transmembrane 4A	-0.280	6296	4581	4116	0.654
Slc39a10	solute carrier family 39 (zinc transporter), member 10	-0.207	6045	3171	563	0.093
Cd164	CD164 antigen	-0.467	5830	4152	4280	0.734
Acox1	acyl-Coenzyme A oxidase 1, palmitoyl	-0.263	5419	7391	4379	0.808
Dip3b	Dip3 beta	-0.240	5062	3013	2063	0.408
Sox6	SRY-box containing gene 6	-0.243	4976	2260	204	0.041
Pcdh17	protocadherin 17	-0.250	4655	3068	2564	0.551
Nr2c2	nuclear receptor subfamily 2, group C, member 2	-0.460	4217	3074	2175	0.516
Ank	progressive ankylosis	-0.090	3934	5379	10433	2.652
Clasp1	CLIP associating protein 1	-0.220	3794	1710	776	0.205
Ube3a	ubiquitin protein ligase E3A	-0.210	3762	5862	4630	1.231
Smad4	MAD homolog 4 (Drosophila)	-0.240	3331	2413	1855	0.557
Tanc2	tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 2	-0.170	3326	1142	331	0.100
Aff4	AF4/FMR2 family, member 4	-0.157	3183	3201	2971	0.934
B230380D07Rik	RIKEN cDNA B230380D07 gene	-0.160	3121	1944	1024	0.328

Table S4. Top 25 miR-219 target genes: OL repressed genes, related to Fig. 6. Top miR-219 predicted target genes, ranked by repression: MOG+ OL / OPC expression level. Genes selected for further study are highlighted.

Supplemental Experimental Procedures

Breeding of mice

Mice homozygous for an allele of *Dicer1* in which the exon coding for most of the second RNAseIII domain has been flanked by LoxP sites (Harfe et al., 2005) were crossed to mice heterozygous for a full-length Cre recombinase insertion into either the *Olig2* or *CNP* gene locus (Lappe-Siefke et al., 2003; Schuller et al., 2008). Mice were crossed for two generations to generate mutant $Olig2^{Cre/+}$ $Dicer1^{flox/flox}$ or $CNP^{Cre/+}$ $Dicer1^{flox/flox}$ mice and associated *Olig2 or* $CNP^{+/+}$ $Dicer1^{flox/flox}$ and *Olig2 or* $CNP^{Cre/+}$ $Dicer1^{flox/+}$ control genotype littermates. See supplemental experimental procedures for genotyping primers. All animal handling protocols were approved by the Stanford University Administrative Panel on Laboratory Animal Care.

Purification and culturing of cells

As described previously (Dugas et al., 2006), OPCs were purified by immunopanning: following negative selection on Ran-2 and GC antibody coated immunopanning plates, OPCs were sorted from enzymatically-dissociated brains by positive selection on an O4-antibody coated immunopanning plate. For isolation of OPCs from transgenic mice, PDGFRα-antibody coated plates (rather than O4 plates) were used for positive selection, as described previously (Cahoy et al., 2008). Purified OPCs were cultured on poly-D-lysine (pDL) coated glass coverslips (for immunostaining) or tissue culture plastic (for all other experiments) in previously described DMEM-Sato based, serum-free medium, which contained or lacked 40 ng/ml T3 ("±T3") and contained or lacked 10 ng/ml PDGF-AA + 1 ng/ml NTF3 ("±PDGF") as indicated. 2% custom B-27

lacking T3 (Chen et al., 2008) was added to all mouse OPC cultures to improve health. All cultures were maintained at 37˚C in 10% CO_2.

Electron microscopy

For EM, optic nerves were dissected, diced into 2-3mm long pieces, and processed as described in Emery et al. (2009). Ultrathin sections were generated either longitudinally (to visualize paranodal structures) or crosswise (to quantify myelinated axons). All images were collected and scored blind; >350 axons / animal were quantified.

Array hybridization

Total RNA samples isolated from cultured immature OPCs and differentiating OLs were 3' end labeled with either Cy3 or Cy5 dinucleotide (CU) using T4 RNA ligase (New England Biolabs) in labeling buffer (50 mM HEPES, pH 7.8, 0.1 mM ATP, 3.5 mM DTT, 20 mM MgCl2, 10 mg/ml BSA). Labeling reactions were incubated at 4˚C overnight, combined (OPC-Cy3 with OL-Cy5 or OPC-Cy5 with OL-Cy3), ethanol precipitated, and resuspended in hybridization buffer (400 mM Na2HPO4 pH 7.0, 0.8% BSA, 5% SDS, 12% formamide). Samples were heated to 95˚C for 3 minutes and hybridized to a microarray at 37˚C for 1-2 hours. Following hybridization, microarrays were washed once with 2X SSC / 0.025% SDS, twice with 0.8X SSC, once with 0.4X SSC, and dried. To control for any potential probe specific effects, the Cy3 and Cy5 labels were swapped and an additional set of arrays were analyzed. In total, 4 arrays were hybridized with OL Cy3-RNA and OPC Cy5-RNA and 4 with OL Cy5-RNA and OPC Cy3-RNA. All microarrays were scanned using GenePix 4000b (Molecular Devices) with PMTs set to 750 (635 channel) and 650 (532 channel). Array data were fit to a linear model using limmaGUI (Wettenhall and Smyth, 2004).

Northern Blotting

Total RNA was purified from acutely purified P23 *Olig2*$^{Cre/+}$ *Dicer1*$^{flox/+}$ and *Olig2*$^{Cre/+}$ *Dicer1*$^{flox/flox}$ OLs using Qiagen miRNeasy Mini Kit. Equivalent amounts of RNA were loaded onto a 15% TBE-Urea PAGE gel, separated by electrophoresis, and transferred to Hybond N+ membrane (Amersham), following manufacturer's instructions (Bio-Rad). Blots were probed with ^{32}P end-labeled LNA probes for miR-219 and then U6 snRNA (loading control) obtained from Exiqon, following manufacturer's instructions.

Genotyping, RT-PCR, and cloning primers

For genotyping, the following primers were used:

Dicer1:

Forward - CCTGACAGTGACGGTCCAAAG

Reverse - CATGACTCTTCAACTCAAACT

wt: 351 bp; floxed allele: 420 bp

Cre recombinase:

Forward - GCTAAGTGCCTTCTCTACACCTGC

Reverse - GGAAAATGCTTCTGTCCGTTTG

PCR recognizes Cre recombinase insertion

For cloning, the following primers were used to amplify the 3' UTR regions of indicated genes:

FoxJ3 (human):

Forward - CACTCAGCACTCCAGGAACA

Reverse - TCAGTTCTGGCAGCAACAAT

PDGFRα (mouse):

Forward - TAAAAAGAGCTCAACCGTGAGTAGTAGGTTTGGGTC

Reverse – AGTGCACTCGAGAACGTGCCTGTGGGGAATATC

Sox6 (mouse):

Forward - AAAACAACGCGTCATTTCGCTCCCTTTCCCCC

Reverse - AATGCTCTCGAGACAACATTCTACCTATGCAGCACAG

ZFP238 (mouse):

Forward - TTCCTGGAACTGAAAAGTTGG

Reverse - AAAAATTCACCAACACACCAA

For standard / quantitative RT-PCR, the following primers were used:

Actin (mouse/rat):

Forward - GCATTGTCACCAACTGGGACG

Reverse - ACCGCTCATTGCCGATAGTG

Dicer1 (mouse), standard RT-PCR:

Forward - ACCAGCGCTTAGAATTCCTGGGAG

Reverse - GCAGCAGACTTGGCGATCCTGTAG

Note: the 5' primer is in the exon deleted by Cre recombinase, 3' primer in the downstream exon

Dicer1 (mouse), qRT-PCR:

Forward - ACCAGCGCTTAGAATTCCTGGGAG

Reverse - GCTCAGAGTCCATTCCTTGC

Note: both primers are in the exon deleted by Cre recombinase

MBP (mouse)

Forward - TGGCAGTGCCCATTGGTACAC

Reverse - TAGAGGGATAAGGAGGTGTCG

FoxJ3 (rat), standard RT-PCR:

Forward - CAGTGTCCTTGCTGTTGTTGGTAAC

Reverse – AGTCATCTCATCTTGCCTGGTCC

FoxJ3 (rat), q RT-PCR:

Forward - TCAGTTCTTCACACAGACGGGC

Reverse - TATGAGGATAACCAGGGGGTGG

MBP (rat), standard RT-PCR:

Forward - CACACACAAGAACTACCCACTACGG

Reverse - AAACAAAGGAAGCCTGGACCAC

MBP (rat), qRT-PCR:

Forward - TTCTTTAGCGGTGACAGGGGTG

Reverse - GACTACTGGGTTTTCATCTTGGGTC

PDGFRα (rat), qRT-PCR:

Forward - CGTCTGGTCTTATGGCGTTCTG

Reverse - TCTCTTTTCGGGTTCACTGTTCC

Sox6 (rat), qRT-PCR:

Forward - TGGTATGAAGATGGACGGCG

Reverse - TGTTGTTGTTGGGGAAAGGGAG

ZFP238 (rat), standard RT-PCR:

Forward - AGGTGGAGAAAGAGGCATCCTG

Reverse - TTGGAGACATAGGGAAGCAGACTG

ZFP238 (rat), qRT-PCR:

Forward - TGAAGACGAAGGCGAAGATGAC

Reverse - AGGGGCTGGCTACTGTTTTTCC

For TaqMan qRT-PCR detection of miRNA expression, the following primer pairs were ordered from Applied Biosystems:

hsa-mir-22 - 4373079

hsa-mir-26b - 4373069

hsa-mir-29a - 4373065

hsa-mir-30c - 4373060

hsa-mir-103 - 4373158

hsa-mir-138 - 4373175

hsa-mir-192 - 4373108

hsa-mir-213 - 4373086

hsa-mir-219 - 4373080

hsa-mir-338 - 4373043

hsa-mir-361 - 4373035

hsa-mir-373 - 4378073

U6 (RNU6B) - 4373381

miRNA reagents

The following miRNA mimic or inhibitor reagents were ordered from Dharmacon:

rno-mir-138 mimic - C-320370-05

rno-mir-219-5p mimic - C-320418-03

rno-mir-338 mimic – C-320259-03

negative control mimic - CN-001000-01

rno-mir-138 inhibitor - C-320370-05

rno-mir-219-5p inhibitor - IH-320418-05

rno-mir-338 inhibitor - IH-320259-05

negative control inhibitor - IN-001005-01

Plasmids

Immunostaining experiments. pC1-eGFP (Clontech 6084-1) was used to mark transfected cells with CMV-promoter driven eGFP production. pSPORT6-FoxJ3 was ordered from OpenBiosystems (MHS1010-99823268), in which a full-length copy of the human FoxJ3 cDNA is driven by a CMV promoter. pSPORT6-ZFP238 is the mouse full-length cDNA (OpenBiosystems MMM1013-98479034) cloned into the EcoR1 (5') and NotI (3') sites in the pSPORT6 vector (FoxJ3 excised, ZFP238 inserted). ZFP238 contains an internal EcoRI site, so the larger EcoRI-NotI fragment was cloned first, then the remaining EcoRI-EcoRI site was inserted to rebuild the entire ZFP238 cDNA.

Luciferase experiments. pRL-TK (Promega E2241) expresses the Renilla luciferase gene from the constitutive HSV-TK promoter. All "test" vectors contain 3'UTRs of candidate genes cloned into pGL3-target, a modified pGL3 vector containing a multiple cloning site downstream of the firefly luciferase gene, which is driven by an SV40 promoter. Primers listed above were used to PCR amplify (using Platinum Taq HiFi, Invitrogen) and clone the following 3'UTRs:

- *FoxJ3*: Amplify 1884-5146 from pSPORT6-FoxJ3 cDNA insert (NCBI BC151828), adding MluI and XhoI sites 5' and 3', respectively, for cloning into pGL3-target.
- *PDGFRα*: Amplify 4036-6326 from the full-length mouse PDGFRα cDNA NM_011058 (this region contained in OpenBiosystems EMM3836-9478919 EST clone NCBI BG078820, insert is full PDGFRα 3'UTR) adding SacI and XhoI sites 5' and 3', respectively, for cloning into pGL3-target.
- *Sox6*: Amplify 2692-4915 from the full-length mouse Sox6 cDNA (NCBI BC067407; OpenBiosystems MMM1013-98478819), adding MluI and XhoI sites 5' and 3', respectively, for cloning into pGL3-target.
- *ZFP238*: Amplify 1953-3905 from full-length mouse ZFP238 cDNA insert (NCBI BC054742), adding MluI and XhoI sites 5' and 3', respectively, for cloning into pGL3-target.

Site-directed mutagenesis

Site-directed mutagenesis was performed with the Stratagene QuikChangeII Site Directed Mutagenesis kit according to manufacturer's instructions, using the following primers:

FoxJ3-

5' primer

CCAATGTTATCAAAATTACTTTTGAAGCCGCGGAGAAGGATTTTAGCTGGAT

AACTTACTGG

2134-2195 from FoxJ3 (human) 3'UTR

2160-2167 219 site changed: GACAATCA to GcCgcggA (change to SacII site)

3' primer

CCAGTAAGTTATCCAGCTAAAATCCTTCTCCGCGGCTTCAAAAGTAATTTTGA

TAACATTGG (reverse complement of 5' primer)

PDGFRa-

5' primer

CTTTCCTGCTTTCTATTTTTATGATGCCGCGGAAAGCTTGCCCGAGGAACACA

ATTTGTG

6037-6096 from PDGFRa 3' UTR

6062-6069 miR-219 site changed: GACAAUCA to GcCgcggA (SacII site inserted)

3' primer

CACAAATTGTGTTCCTCGGGCAAGCTTTCCGCGGCATCATAAAAATAGAAAG

CAGGAAAG (reverse complement of 5' primer)

Sox6-

5' primer

GTCTTACAGGAGACACTGCAAAGTCTTAGCCGCGGTTTGACATCTTAAAATA

AAACAGC

3256-3314 from Sox6 (mouse) 3'UTR

3284-3290 miR-219 site changed: GACAATC to GcCgcgg (SacII site added)

3' primer

GCTGTTTTATTTTAAGATGTCAAACCGCGGCTAAGACTTTGCAGTGTCTCCTG

TAAGAC (reverse complement of 5' primer)

ZFP238-

5' primer #1 (SacII)

GTTAAAATAATCTTTATTAATCTAGAGGAGCCGCGGAATGTCTGTGGAAGAA

CGGACTTTTTTGG

3659-3723 from ZFP238 (mouse) 3'UTR

3688-3695 miR-219 site changed: GACAAUCA to GcCgcggA (SacII site inserted)

3' primer #1 (SacII)

CCAAAAAAGTCCGTTCTTCCACAGACATTCCGCGGCTCCTCTAGATTAATAAA

GATTATTTTAAC (reverse complement of 5' primer)

5' primer #2 (PvuII)

GTTCTAAATCTTAGACTGACATCTAGCTTTGCAGCTGATAGTATGTTTTATTTC

CTGAGGGGG

3791-3853 from ZFP238 (mouse) 3'UTR

3821-3828 miR-219 site changed: GACAATCA to GcagcTgA (insert PvuII site)

3' primer #2 (PvuII)

CCCCCTCAGGAAATAAAACATACTATCAGCTGCAAAGCTAGATGTCAGTCTA

AGATTTAGAAC (reverse complement of 5' primer)

Luciferase assays and 293 cell transfections

HEK-293 cells were cultured in DMEM + 10% fetal calf serum (Invitrogen) and grown to 90-95% confluence, then transfected with Lipofectamine 2000 (Invitrogen) following manufacturer's instructions. For luciferase assays, 1 µg test firefly luciferase plasmid, 0.15 µg pRL-TK Renilla luciferase control plasmid (Promega), and 25 pmol

miRNA mimic reagent were combined in transfections. Cells were then cultured 24-48 hours at 37˚C, 5% CO_2.

Luciferase activity was assayed using Dual-Luciferase Reporter Assay System (Promega). In all experiments, firefly (test) luciferase activity was normalized relative to the level of Renilla (transfection control) luciferase activity in the same well. Within a single experiment (comparing miR-219 mimic to control reagent), all individual firefly/Renilla ratios were then normalized to the average of the control mimic - test firefly - control Renilla transfection well ratios.

Cell survival assays

Cell survival in *FoxJ3* and *ZFP238* transfections was determined after 2 DIV in proliferative (+PDGF-T3) or differentiation (-PDGF-T3) media, using the Invitrogen LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells, following manufacturer's instructions.