Cell Reports, Volume *29*

Supplemental Information

Oligodendrocyte Intrinsic miR-27a Controls Myelination and Remyelination

Ajai Tripathi, Christina Volsko, Jessie P. Garcia, Eneritz Agirre, Kevin C. Allan, Paul J. Tesar, Bruce D. Trapp, Goncalo Castelo-Branco, Fraser J. Sim, and Ranjan Dutta

Supplemental items:

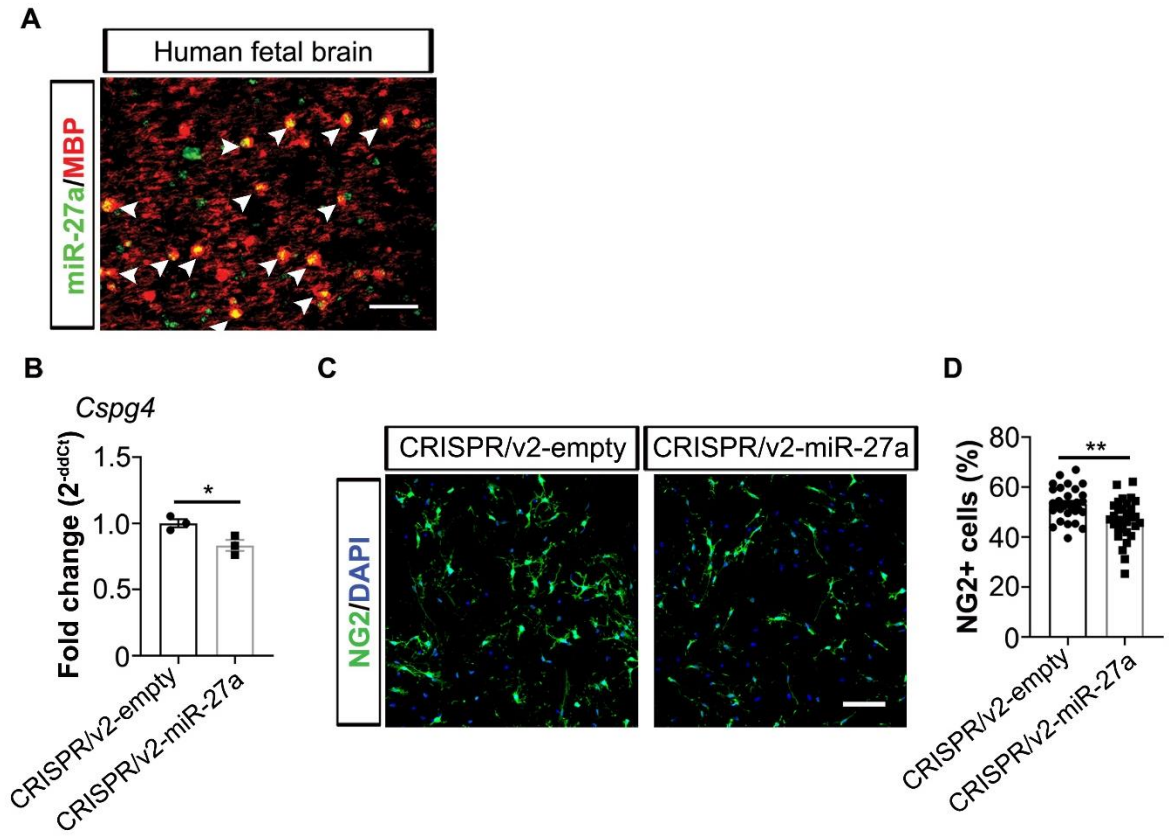


Figure S1 (related to Figure 1): Importance of miR-27a in OPC lineage development

(A) Representative immune-*in situ* hybridization images showing miR-27a (green) and MBP (red) staining of human fetal brain. Scale bar- 50µm.

(B) qPCR analysis of *Cspg4* levels in CRISPRv2-empty and CRISPRv2-miR-27a KO mouse EpiSCs-derived OPCs. *Gapdh* was used as an internal control for qPCR analysis. Data represent mean ± SEM fold change of 3 independent experiments; **P*<0.05; Student's *t*-test, two-sided.

(C) Representative images (from three independently repeated experiments with similar results) of CRISPR/v2 empty (left) and CRISPR/v2-miR-27a KO (right) mouse EpiSCs-derived OPCs showing NG2 expression (right). Scale bar- 100µm.

(D) Percentage of NG2+ cells in CRISPRv2-empty and CRISPRv2-miR-27a KO mouse EpiSCs-derived OPCs in proliferation media. Data represent mean ± SEM of NG2+ cells from 3 independent experiments; ** *P*<0.01; Student's *t*-test, two-sided.

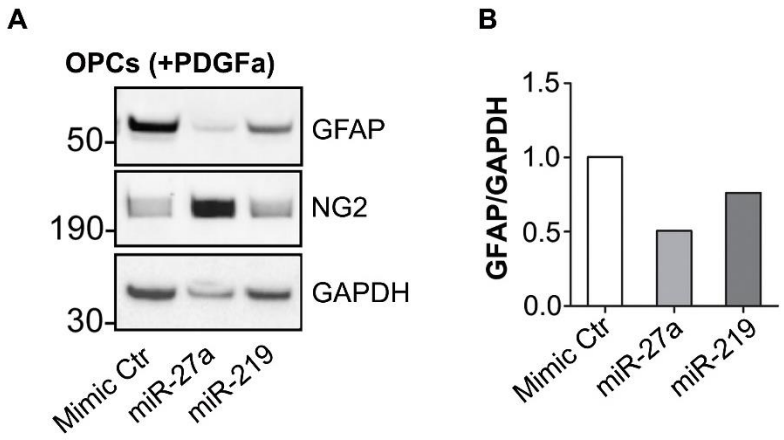


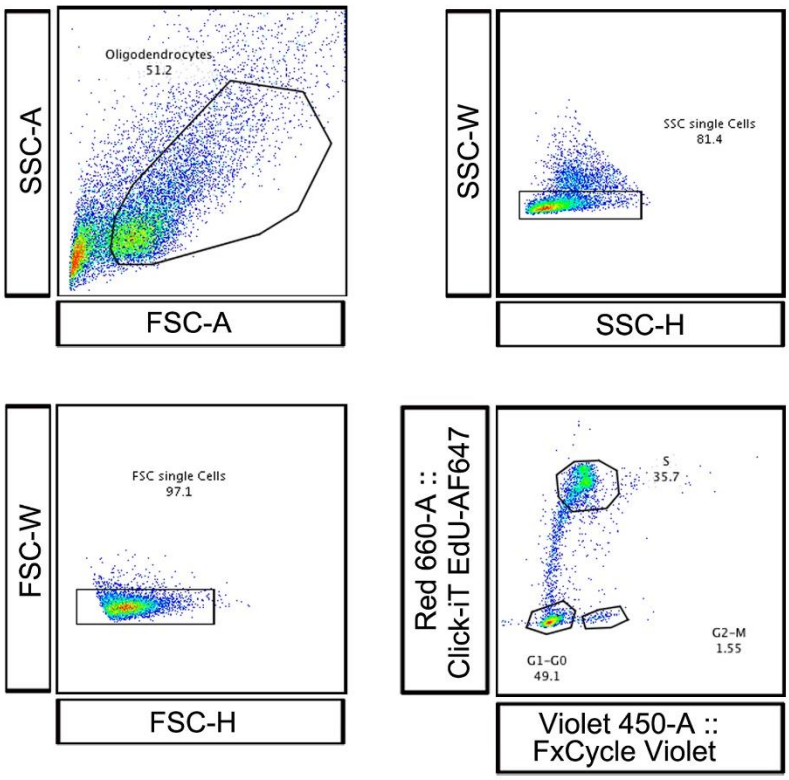
Figure S2 (related to Figure 2): Analysis of GFAP expression in miR-27a-mimic-transfected mouse OPCs

(A) Western blot image of GFAP and NG2 expression in miRNA mimic (Ctr, miR-27a and miR-219)-transfected mouse OPCs. GAPDH expression was used as a loading control.

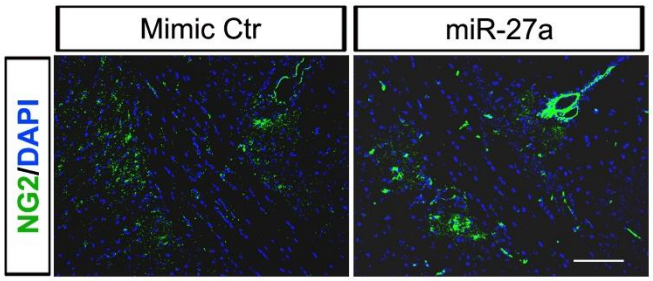
(B) Quantification of GFAP levels from the western blot analysis of miRNA-mimic transfected OPCs.

A

Gating strategy:



B



C

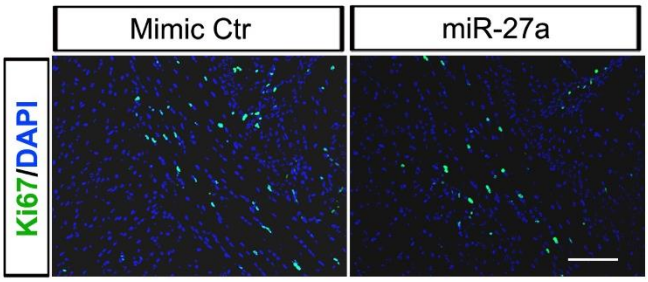


Figure S3 (related to Figure 3): Increased miR-27a inhibits proliferation of OPCs

(A) Flow cytometry gating strategy for cell proliferation assay to analyze cell populations at different stages of the cell cycle.

(B, C) Representative immunohistochemical images (from three mouse brains with similar results) of OPCs (NG2- green, **B**) and proliferating cells (Ki67- green, **C**) in intranasally miRNA mimic (Ctr and miR-27a)-treated P12 mouse brain corpus callosum. Nuclei were stained with DAPI (blue). Scale bar- 50µm.

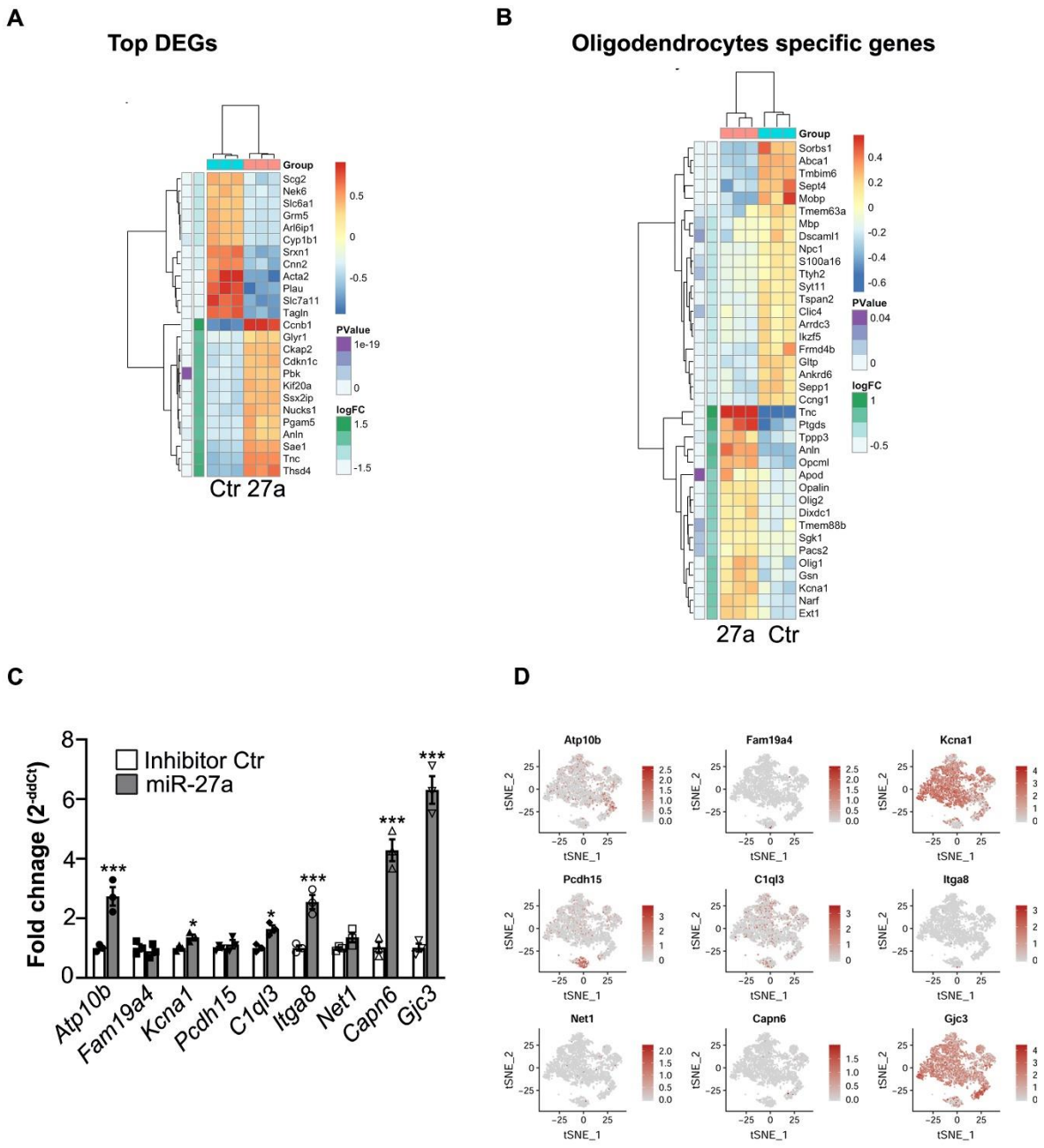


Figure S4 (related to Figure 4): miR-27a targets OL lineage-specific genes

(A) Heatmap showing differentially expressed genes in miR-27a mimic-transfected mouse OPCs.

(B) Heatmap showing upregulated OPC-specific genes and decreased levels of mature OL stage-specific genes in miR-27a mimic-transfected OPCs.

(C) qPCR analysis of *Atp10b*, *Fam19a4*, *Kcna1*, *Pcdh15*, *C1ql3*, *Itga8*, *Net1*, *Capn6,* and *Gjc3* levels in OPCs transfected with miR-27a inhibitor. *Gapdh* was used as an internal

control for qPCR analysis. Data represent mean ± SEM fold change of 3 independent experiments; *P<0.05, ***P<0.001; Two-way ANOVA with Bonferroni's *post hoc* test, multiple comparisons.

(D) *t*-SNE projection of cells clusters showing selected enriched gene expression during normal OL linage development.

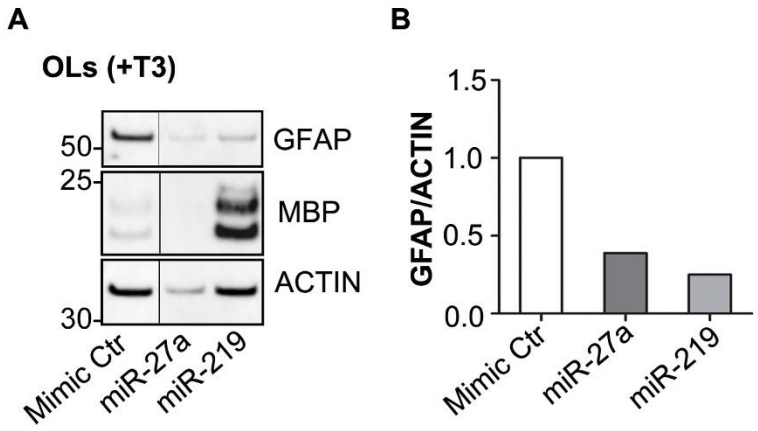


Figure S5 (related to Figure 5): Analysis of GFAP expression in miR-27a-mimic-transfected differentiated mouse OPCs

(A) Western blot analysis for GFAP and MBP expression in miRNA mimic (Ctr, miR-27a, and miR-219)-transfected mouse OLs. Black vertical line represents break in gel loading with a different sample.

(B) Quantification of GFAP levels from western blot analysis of miRNA mimic-transfected OLs.

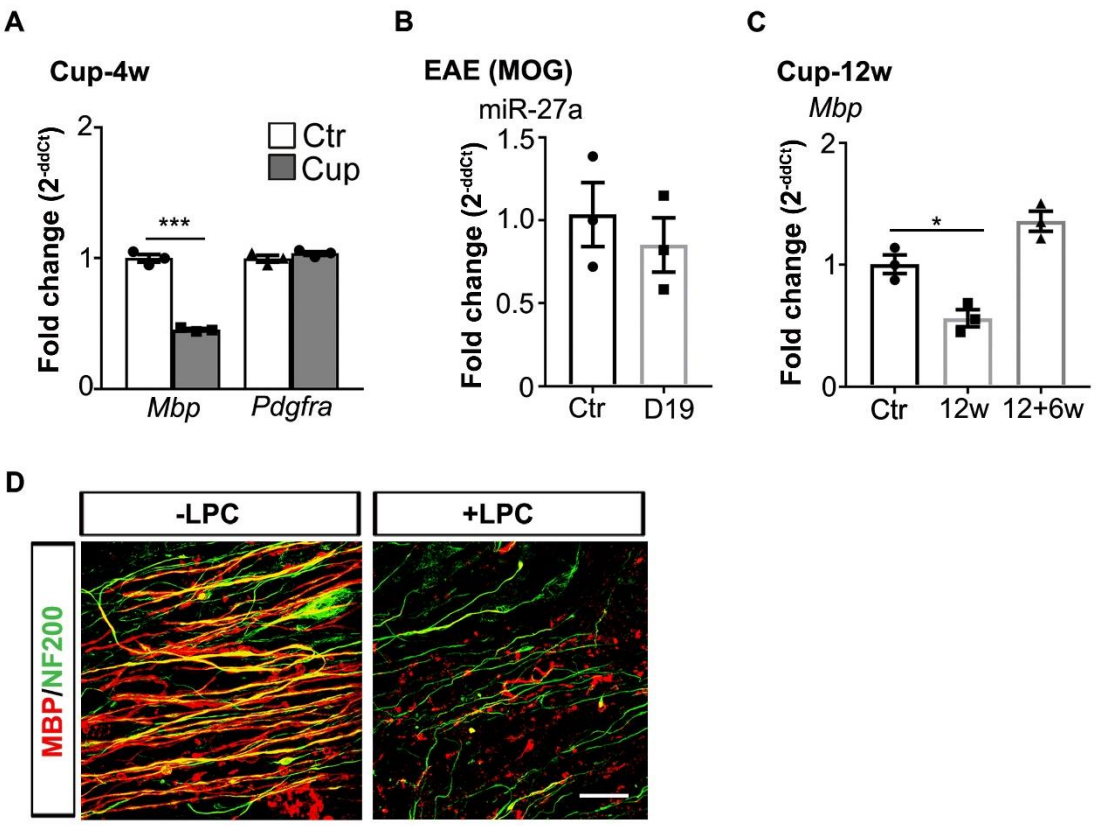


Figure S6 (related to Figure 7): miR-27a expression during demyelination and remyelination

(A) qPCR analysis of *Mbp* and *Pdgfrα* levels in mouse brain corpus callosum (from three different mouse brains) fed cuprizone for 4 weeks (Cup-4w). *Gapdh* was used as an internal control for qPCR analysis. Data represent mean ± SEM fold change from 3 different mouse brains; ***P<0.001; Student's *t*-test, two-sided.

(B) qPCR analysis of miR-27a levels at peak of disease (Day 19- D19) in the spinal cords of EAE mice. U6 snoRNA was used as an internal control for qPCR analysis. Data represent mean ± SEM fold change from 3 different mouse spinal cord tissues; Student's *t*-test, two-sided.

(C) qPCR analysis of *Mbp* levels in mouse brain corpus callosum during demyelination (Curipzone, 12 weeks, Cup-12w) and remyelination (12+6w). *Gapdh* was used as an internal control for qPCR analysis. Data represent mean ± SEM fold change from 3 different mouse brains; *P<0.05; Student's *t*-test, two-sided.

(D) Representative confocal images of myelinated exons (MBP-red, NF200-green) in control and LPC-treated P12 mouse brains, respectively. Scale bar- 20µm.