

Figure 1S: REST is expressed in acutely dissociated rat optic nerve glia. Immunofluorescence staining showing REST protein (green) in dissociated P7 optic nerve glia after 3hr in culture. Antibodies against trypsin resistant cell specific proteins were used to identify different types of glia (red, top to bottom; A2B5-OPCs, O4-pre-oligodendrocytes, GFAP-astrocytes) as indicated and cell nuclei were stained with DAPI (blue). bar=50 μ m.

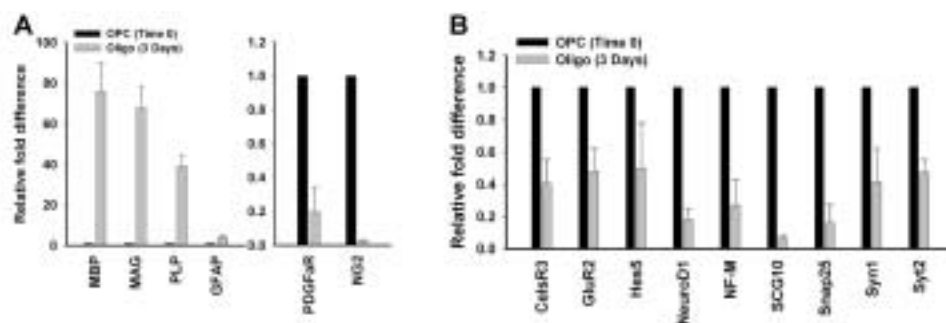


Figure S2: Changes in gene transcription as OPCs differentiate into oligodendrocytes. **A:** 3 days after initiating oligodendrocyte differentiation, myelin gene transcription is increased whereas the transcription of OPC-associated genes is reduced. **B:** Transcription of RER1-containing genes is reduced 3 days after initiating oligodendrocyte differentiation. Data shown is fold difference relative to OPCs from real-time PCR after normalization to GAPDH. Error bars represent the standard deviation from the averages of 2-3 PCR runs from 2 separate experiments.

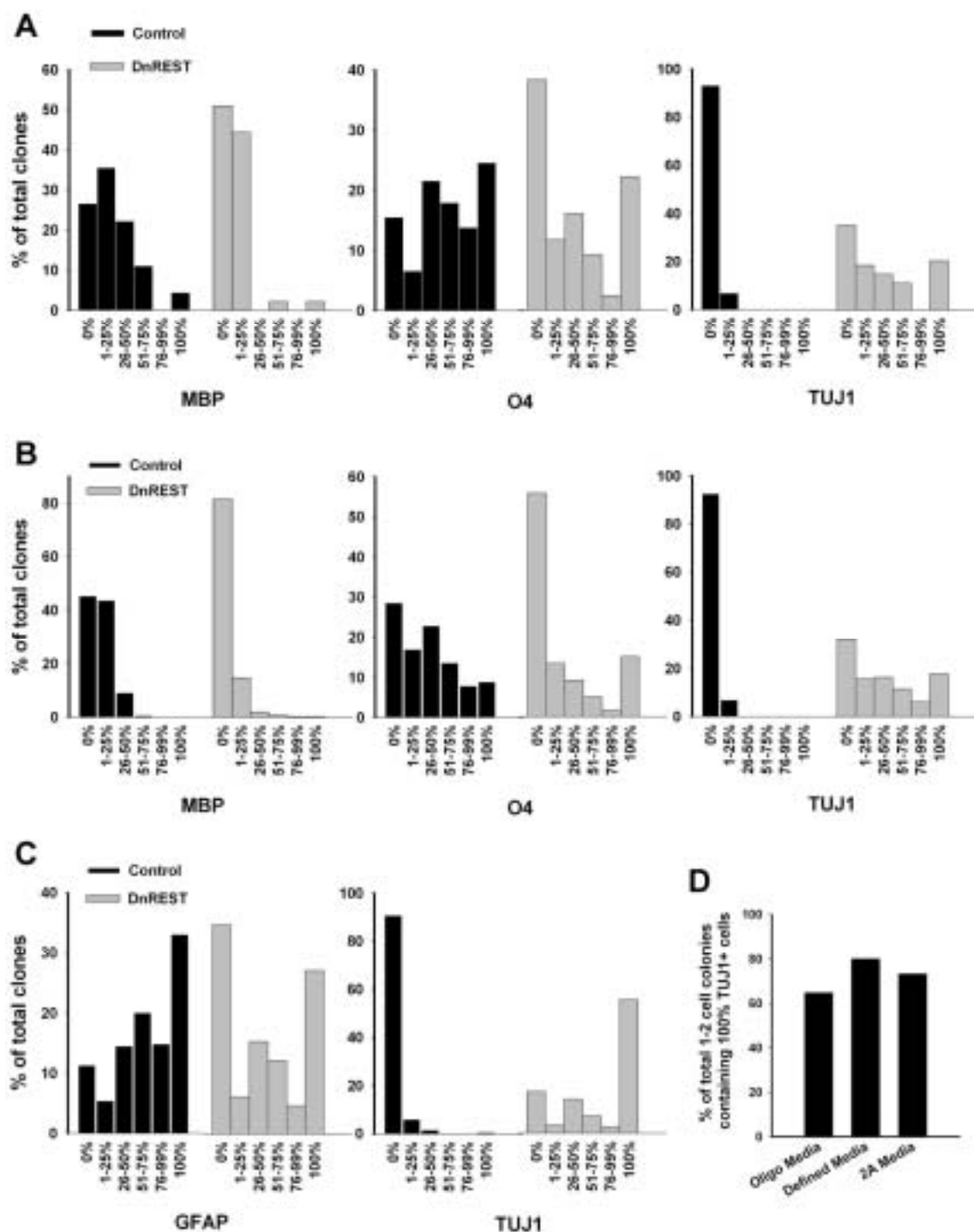


Figure S3 Clonal composition after REST LOF. OPCs were infected and grown at clonal density as described under Materials and Methods and analyzed for the expression of MBP, TUJ1, or GFAP after 5 days or O4 after 3 days in their differentiation media. The histograms show the percent of total clones that contain the indicated percentages of antigen positive cells. Total number of clones analyzed is as follows. **A.** Oligodendrocyte media, MBP: control n= 45 DnREST n= 45, O4: control n= 167, DnREST n= 160, TUJ1: control n= 58, DnREST n = 54. **B.** Defined Media, MBP: control n= 251, DnREST n= 251, O4: control n= 108, DnREST n= 116, TUJ1: control

n=136, DnREST n= 146. **C.** 2A media, GFAP: control n=273, DnREST: n= 279, TUJ1: control n=202, DnREST n= 194. **D.** The graph shows the % of 1 and 2 cell DnREST-infected clones that are 100% TUJ1- positive after 5 days in the indicated media.

**TABLE S1
GENE CHANGES WITH REST LOF**

gene	2A	Oligo
CelsR3	+++	+++
Snap25	+++	+++
Calbindin	-	+
Synapsin	++	++
Synaptotagmin	+	NC
SCG10	++	++
GluR2	NC	+
NFM	++	NC
Pou4F3	+++	++
Type II Na	NC	NC
TrkC	-	NC
NeuroD1	+++	+++
NeuroD2	+++	++
Myt1	+	++
Myt1L	+	NC
Olig1	NC	NC
Olig2	NC	+
Hes1	NC	+
Hes5	+	+
Mash1	-	+
Sox2	NC	NC
Nkx2.2	-	-
DCX	NC	++
Nestin	NC	+
PDGFaR	NC	NC
PLP	NC	NC
GFAP	-	-

+++ >15 fold increase
 ++ 4-15 fold increase
 + 1.5-4 fold increase
 - >1.5 fold decrease
 NC No change

Table S1: Loss of REST repression induces multiple gene changes in glia. Gene transcription was measured by real time PCR in the indicated cell types 72hr after infection with adenoviruses expressing REST-VP16 or GFP as a control. Transcript

levels are normalized to GAPDH and fold changes in gene expression are relative to control infected cells for each cell type. Data is representative of 3 separate experiments. The list of genes includes genes known to be directly regulated by REST, pro-neural and/or pro-glia transcription factors that may or may not be REST regulated, and cell-type specific markers.