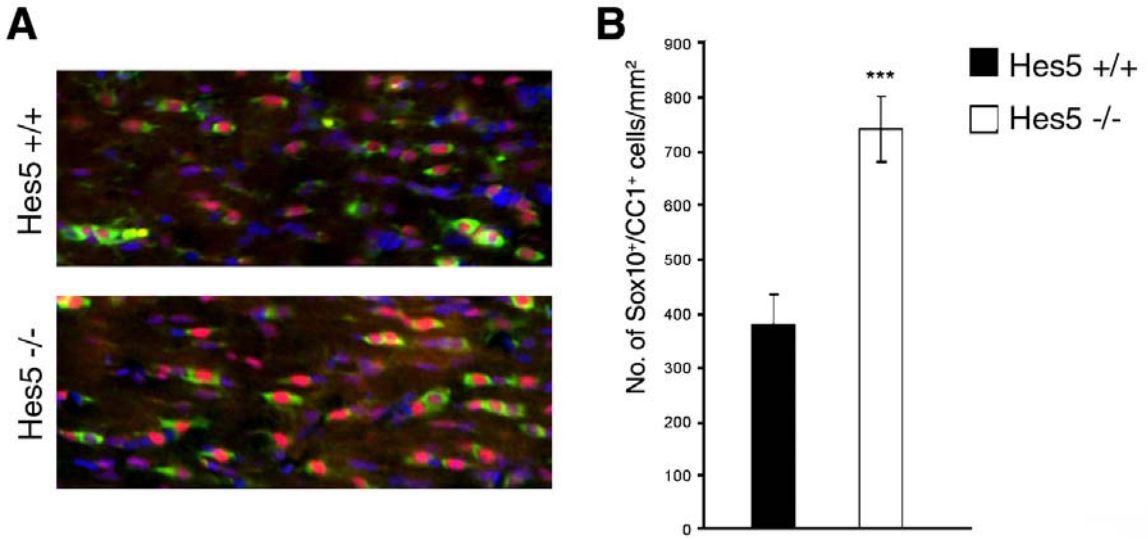
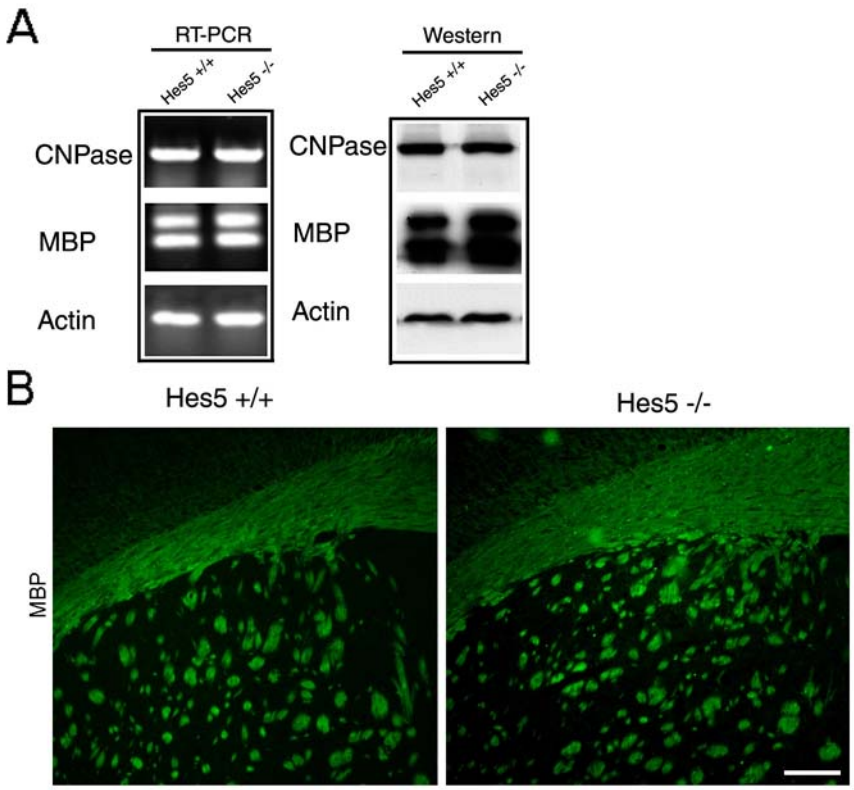
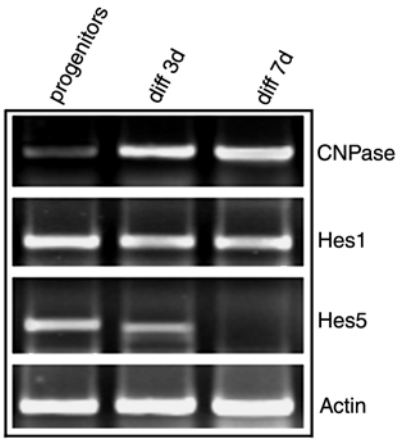


Supplementary Figure 1: Loss of Hes5 function in vivo results in increased number of mature oligodendrocytes in the postnatal brain. Immunohistochemistry of coronal brain cryosections from p11 wt (Hes5 +/+) and mutant (Hes5-/-) mice and stained with antibodies against the oligodendrocyte markers APC, recognized by the antibody CC1 (green in A) and against the transcription factor Sox10 (red in A). DAPI (blue in A) was used as nuclear counterstain. Mature oligodendrocytes were identified by double immunofluorescence for Sox10 and CC1 (A). The number of mature cells was counted in the medial corpus callosum of wild type and Hes5 mutant sections at the same anatomical level and was then normalized per unit surface area. The bar graphs represent the average and standard error of cell counts from at least three section per mouse and at least three mice per genotype (B). Note that the increased number of cells in Hes5 mutants compared to wild type mice was statistically significant (* =$p<0.05$, *** =$p<0.0005$). Scale bar=50 μm.



Supplementary Figure 2: The increased expression of myelin gene transcripts observed in the brain of developing *Hes5-/-* mice is a transient phenomenon and does not persist in adult mice. The levels of myelin gene products were measured in the brain of young adult wild type (*Hes5+/+)* and knockout (*Hes5-/-)* mice. Note that similar transcript levels for the myelin genes CNPase and MBP were detected by RT-PCR in wild type and Hes5 mutants. Actin was used as internal control (A). Protein levels were measured using western blot analysis of extracts from the brain of mice of each genotype and revealed similar levels of CNP and slightly elevated levels of MBP in the mutants (B). Immunohistochemistry using MBP antibodies revealed a similar intensity of staining of myelinated fibers in mice of both genotypes (C).



Supplementary Figure 3: Hes5 expression levels decrease during the differentiation of Oli-Neu cells and are inversely correlated with the levels of myelin genes. RT-PCR of RNA samples isolated from mouse immortalized oligodendrocyte progenitors (i.e. Oli-Neu cells) and amplified with primers specific for the murine (i.e. endogenous) Hes1, Hes5 and for the myelin gene product CNPase. Note the high levels of Hes5 in undifferentiated progenitor cells, their progressive decline in cells differentiated for 3 and 7 day, and the corresponding increase in myelin gene products (i.e. CNPase). The levels of Hes1 were only subtly decreased during differentiation. Actin was used as internal control.

Primers used in RT-PCR

name	forward	reverse
Actin	TGGAATCCTGTGGCATCC	TCGTACTCCTGCTTGCTG
Mash1	CTTCCTTAAGGCCTCTGGCT	GAACCCGCCATAGAGTTCAA
Id1	CTCAGGATCATGAAGGTCGC	AGACTCCGAGTTCAGCTCCA
Id2	TCTCCTCCTACGAGCAGCAT	ATTCAGATGCCTGCAAGGAC
Id3	AACGTAGCCTGGCCATTG	GTCAGTGGCAAAAGCTCCTC
Id4	TGCAGTGCGATATGAACGAC	CAGCTCAGCGGCAGAGAAT
Sox10	GACCAGTACCCTCACCTCCA	CCCCTCTAAGGTCGGGATAG
Olig1	ATGAGCTGGTGGGTTACAGG	CACCAGCTGGGAGAGAGAAC
Olig2	CTGGTGTCTAGTCGCCCATC	CACCAGTCGCTTCATCTCCT
Nkx2.2	GGGTTTTCAGTCAAGGACA	TGTACTGGGCGTTGTACTGC
CNPase	AGACTTCTCCGAGGCGTACA	TCTCTTCACCACCTCCTGCT
UDP-ceramide-galactosyl-transferase	GGAGTGCTGTTGGAATAGCAA	GGCAGCCATTCTATGAGCTTA
MBP	ATGGCATCACAGAAGAGACC	CATGGGAGATCCAGAGCGGC
Mouse Hes1	ACACCGGACAAACCAAAGAC	AGCCACTGGAAGGTGACACT
Mouse Hes5	CAAGGAGAAAAACCGACTGC	GCTGGAAGTGGTAAAGCAGC
Rat Hes1	ACCGGACAAACCAAAGACAG	GAATGTCTGCCTTCTCCAGC
Rat Hes5	GCAGCATTGAGCAGCTGAAAC	TCCTGCAGGCACCACGAGTAA
Pax6	AGTTCTTCGCAACCTGGCTA	ACTTGGACGGGAACTGACAC
YY1	GGTGCAGATCAAGACCCTGGA	GTGTGCGCAAATTGAAGTCCA