

Supplementary Data #S5:

Material and methods section:

Western-blots. Western blots were performed as described previously with typically 20-40 μg of total cell extract run on SDS gels (Boutillier et al., 2003). Specific bands were detected by enhanced chemiluminescence (Amersham, Little Chalfont, UK). Blots were reprobbed for beta-actin, which served as a control for gel loading.

Isolation of E2F-RE binding proteins. We used the μMACS Streptavidin Kit (Miltenyi Biotec GmbH, Germany) for binding assays. Nuclear protein extracts from CGN were prepared according to Dignam et al. (1983). Biotinylated DNA (1 μg) was incubated with 400 μg of nuclear protein extracts in the presence of 20 μg polydIdC in the binding buffer (10mM HEPES, pH 7.9, 6mM KCl, 4 mM MgCl_2 , 5mM DTT, 8% glycerol, 2% ficoll, 100mM NaCl, protease and phosphatase I/II inhibitors cocktail). μMACS Streptavidin MicroBeads were then added. This mixture was applied to a μColumn where the MicroBeads and specifically bound proteins were retained in the magnetic field of a μMACS Separator. Non-specifically bound molecules were removed by 4 steps of stringent washes (10mM HEPES, pH7.9, 6mM KCl, 4mM MgCl_2 and 200mM NaCl). Target bound proteins were then eluted by buffer containing 10mM HEPES, pH7.9, 4mM KCl, 4mM MgCl_2 and 1M NaCl. All fractions were analyzed by western blot.

Immunoprecipitation. Cultured CGN ($1 \cdot 10^6$ cells) were used for each immunoprecipitation reaction. After lysis (25mM tris (pH 7,4), 200mM NaCl, 1mM EDTA, 0,01 % SDS, 0,5% Triton X-100, 10% glycerol, protease inhibitors cocktail), cell extracts were precleared and incubated with 5 μg of appropriate primary antibody overnight at 4°C, followed by 2h incubation with 40 μl of protein A or G. The beads were washed 5 times with the same buffer and boiled in 40 μl of sample buffer before Western blot analyses.

In vivo Chromatin immunoprecipitation. Briefly, cerebelli were dissected from mice, bladed and 1% of formaldehyde was added for cross-linking during 10 min. Tissue was disaggregated with a dounce homogenizer and cell pellet was lysed on ice. Chromatin was sonicated and then processed as described for *in vitro* ChIP experiments. PCR were run with

primer sets previously described: *e2f1* (Boutillier et al., 2003), *dhfr* (Ferreira et al., 2001), *cyclin A* (Ghosh and Harter, 2003).

Reverse transcription-polymerase chain reaction analyses. Total RNA was extracted from neurons using the RNeasy Mini kit purchased from Qiagen (Valencia, CA, USA) according to manufacturer's procedures. A 2 μ g aliquot was reverse-transcribed using 200 U of MMLV reverse transcriptase (Promega, Madison, WI, USA) and PCR analysis were performed as described previously (Boutillier et al., 2003).

RT-PCR analysis primers:

vgcl, FOR: 5'-CGTTCTCATCCTGCTCAACA-3' and REV: 5'-GGTGTACCTCGGTGATTGCT-3'; *map2*, FOR: 5'-TGGTCCTTGTATTGGGCTTC-3' and REV: 5'-CAGGTCACAGGGCACCTATT-3'; *cyclin A*, FOR: 5'-GTCTCTTTACCCGCAGCAAG-3' and REV: 5'-TCTTCTCCCACCTCAACCAG-3'; *dhfr*, FOR: 5'-TTCTGCCTGGCCTTGTAAT-3' and REV: 5'-CCCAGCTGAAGAGCTATTGG-3'; *Bmyb*, FOR: 5'-CACCCCTGCACCAGAAGTAT-3' and REV: 5'-GAGACTTGCGGACCTTCTTG-3'; *HP1 α* , FOR: 5'-CCCTGGGCTTATTGTTTTCA-3' and REV: 5'-GGTTAAGGGGCAAGTGAAT-3'; *HP1 β* , FOR: 5'-GTCAAGGGCAAGGTGGAATA-3' and REV: 5'-GCACTTGACATTGGCTTCCT-3'; *HP1 γ* , FOR: 5'-CAG AGG TCT CGA CCC TGA AC-3' and REV: 5'-GCT CCT CGT AGA AGG CAA TG-3'. *e2f1*, *cyclin E* and *gapdh* primers are described elsewhere (Boutillier et al. 2003).

Determined semi-quantitative PCR conditions were as follows: 28 cycles for *e2f1*, *cyclin E*, *cyclin A*, *dhfr*, *Bmyb*, *p21* and *gapdh* (each cycle of PCR consisting of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C), 24 cycles for *map2* and 26 cycles for *vgcl*. To ensure linear signal generation, the control cDNA was diluted (1/2 and 1/4) or not and amplified within the same experiment.

DNase I sensitivity assay:

Digestion buffer: 60mM KCl, 15mM NaCl, 15mM Tris-HCl, pH 7.4, 3mM MgCl₂, 0.5mM dithiothreitol, 0.25M sucrose.

Primers: *e2f1*, *cyclin A*, *p21*: same as in ChIP primers.

myogenin: FOR: 5' -GATCACATGTAATCCACTGGA-3', REV: 5'-ACGCCAACTGCTGGGTGCCA-3'

gapdh: Fereirra et al. (2001).

Quantifications and statistics. The intensity of bands obtained by western blot or PCR was analyzed with the NIH Image software and relative amounts or fold inductions are presented as noted. Experiments are performed at least 3 times in duplicates or triplicates and histograms represent the mean values \pm SEM. Statistical significance was determined by one-way ANOVA followed by Newman-Keuls' multiple comparison test. Difference was considered as significant at $P < 0.01$. Typically, a representative experiment is shown on figures, unless otherwise noted.