Supplemental Figures and Supplemental Table

Figure S1. Analysis of cell cycle kinetics in H2-b2T neuroepithelial cells using cumulative labeling with BrdU. A) DNA-specific staining with bisbenzimide (Bisb.) revealed that the very first mitotic figures showing BrdU incorporation (arrow) were observed 2 h after addition of BrdU. This time point represents the duration of G2 in H2-b2T neuroepithelial cells. The mitotic index (MI) was estimated in these cultures as the percentage of cells showing mitotic figures. This value was used to estimate the duration of mitosis in H2-b2T neuroepithelial cells (see Methods). Arrowhead points to a mitotic figure lacking BrdU. B) Scheme illustrating the BrdU labeling procedure used to quantify of the duration of the cell cycle (T_S) and the S-phase (T_S) in asynchronic cells, as described by (Takahashi et al., 1993). At the beginning of the BrdU treatment (t_0) only those cells undergoing S-phase become labeled with this nucleotide analogue (red nucleus in cell 1). The fraction of cells that become labeled with BrdU (i.e. labeling index, LI) gradually increases as the cells progress throughout the cell cycle and undergo S-phase (cell 4 in t_1 and cell 3 in t_2). This is indicated by the progressive length expansion of the red stripes as time increases. The time point at which all proliferating cells become BrdU labeled (t_{GF}) represents T_C minus T_S. After t_{GF} LI remains constant over time, and this LI value is referred to as the growth fraction (GF), which represents the fraction of proliferating cells in the culture. The fraction of cells incorporating BrdU at t_0 along with the values of GF and t_{GF} can be used to estimate T_C and T_S (see Materials and Methods). C) Estimation of the proportion of H2-b2T neuroepithelial cells that incorporate BrdU after the indicated time points (n=3). A plateau was reached after 17 h, and the y-intercept was observed to occur at a LI value of around 0.3. These values were used to estimate T_C and T_S (see Materials and Methods). (D) Diagram illustrating the estimation of the duration of G1, S, G2 and M throughout the cell cycle in H2-b2T neuroepithelial cells, as defined by the LI profile shown in (C) and the analysis described in (A).

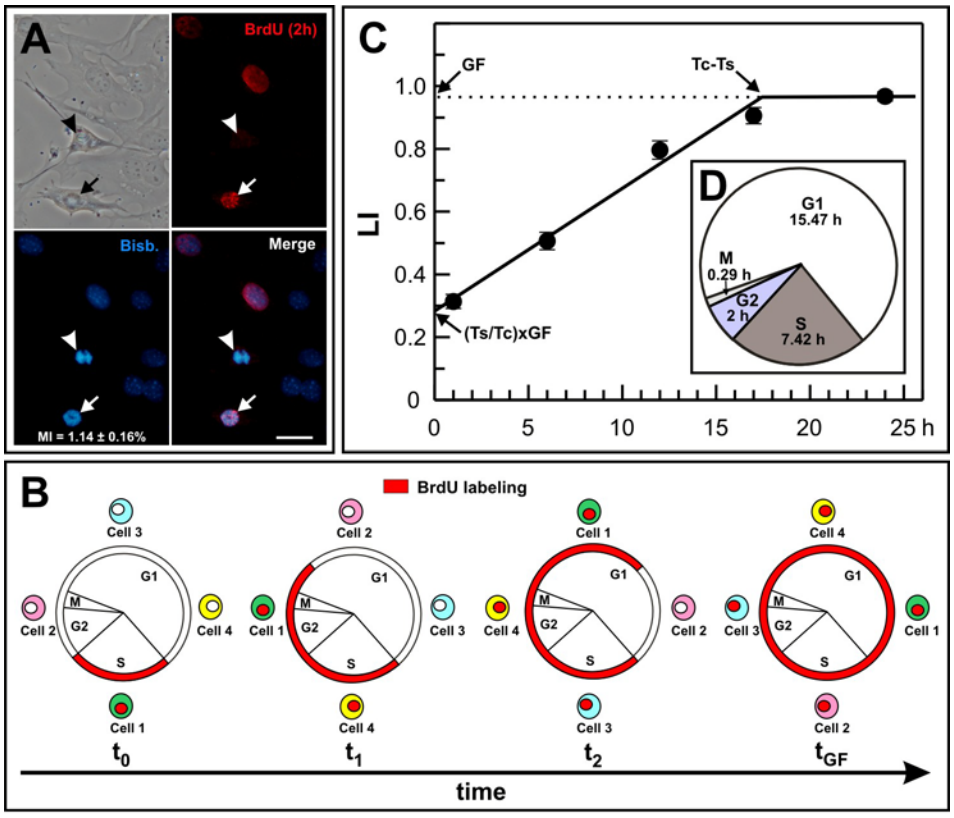
A
BrdU (2h)
Bisb.
Merge
MI = 1.14 ± 0.16%
C
GF
Tc-Ts
(Ts/Tc)xGF
LI
1.0
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25 h
D
G1
15.47 h
M
0.29 h
G2
2 h
S
7.42 h
B
BrdU labeling
Cell 1
Cell 2
Cell 3
Cell 4
G1
G2
M
S
t0
t1
t2
tGF
time

Fig. S1

Figure S2. Most H2-b2T neuroepithelial cells are octoploid. The amount of DNA in either H2-b2T neuroepithelial cells (upper panel) or E15 telencephalic precursors (lower panel) was revealed by flow cytometry after labeling with propidium iodide. Diploid telencephalic precursors showed two main peaks representing cells in G1/G0 (2C DNA content) or in G2 (4C DNA content). In contrast, most H2-b2T neuroepithelial cells displayed a profile typical from proliferating octoploid cells.

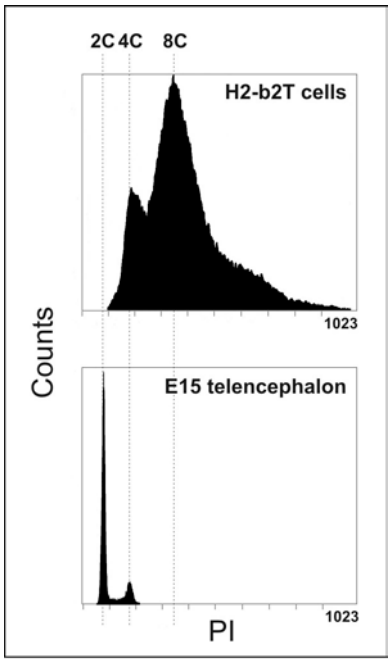

Fig. S2

Figure S3. Expression of Elavl1/HuR in the H2-b2T neuroepithelial cells. A) Parental control H2-b2T cells double immunostained for Elavl1/HuR (HuR) or phospho-Histone H3 (pH3), and counterstained with bisbenzimide (Bisb.) In most cells strong immunoreactivity for Elavl1/HuR correlated with the presence of mitotic figures (arrows). The majority of these latter cells showed, Elavl1/HuR immunostaining in areas excluded from the DNA. B) ED cells immunostained with antibodies against Elavl1/HuR (red) and counterstained with bisbenzimide (blue). Most cells expressing high levels of Elavl1/HuR showed enhanced expression of EGFP (green, see arrows), while cells with low expression of this RBP barely expressed EGFP (arrowheads). Bar: 6 μm (A); 3 μm (B).

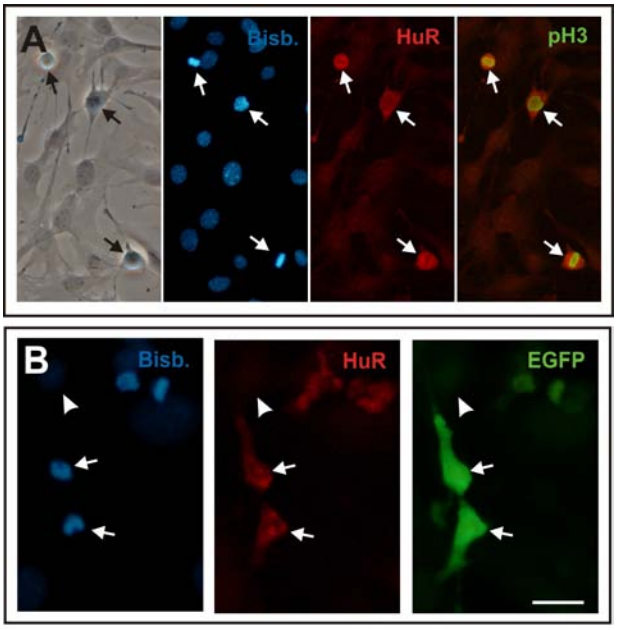

Fig. S3

Figure S4. Expression of Elavl1/HuR, but not Elavl2/HuB, Elavl3/HuC, or Elavl4/HuC, by H2-b2T cells. cDNAs obtained from brain of E10.5 mouse embryos (Brain) or ED cells synchronized in M (H2-b2T) were subjected to PCR amplification with oligonucleotides specific for Elavl2/HuB (HuB), Elavl3/HuC (HuC), Elavl4/HuD, or Elavl1/HuR (HuR). +RT: amplifications carried out in the presence of reverse transcriptase; -RT: amplifications carried out in the absence of reverse transcriptase. Asterisks: primers.

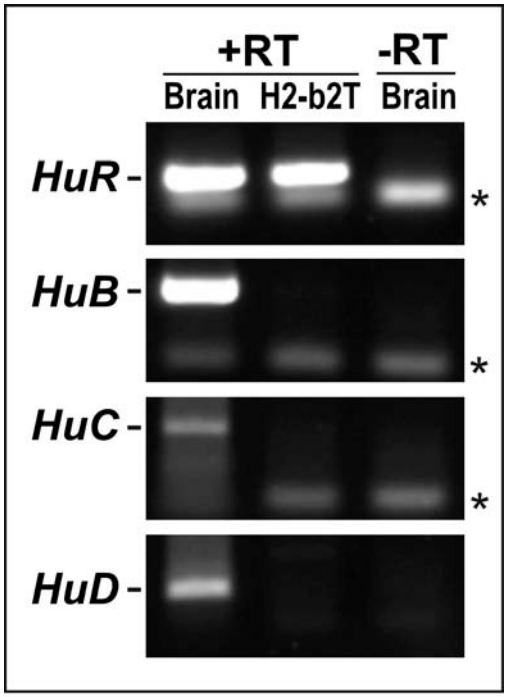

Fig. S4

Figure S5. An *Elavl1*-specific RNAi construct reduces Elavl1/HuR expression in H2-b2T neuroepithelial cells. (A) H2-b2T cells were co-transfected with RFP (Red) plus the *Elavl1*-specific RNAi vector (HuR RNAi) or the pSilencer control vector (Control), synchronized in M with 1 µg/ml colchicine, fixed, and immunostained with an anti-Elavl1/HuR antibody (HuR). The presence of the Elavl1-specific RNAi construct resulted in a clear reduction of Elavl1/HuR immunolabeling in many cells (arrowheads; for a quantification see Figure 6A). (B) H2-b2T cells were co-transfected with RFP plus the *Elavl1*-specific RNAi vector (HuR RNAi) or the pSilencer control vector (Control), synchronized in M with 1 µg/ml colchicine, and subjected to cell sorting to isolate the RFP-positive cells. Cells were then extracted with laemli buffer, and subjected to western blot with antibodies specific for Elavl1/HuR (HuR) or βIII tubulin (βIII tubulin). Bar: 5 µm.

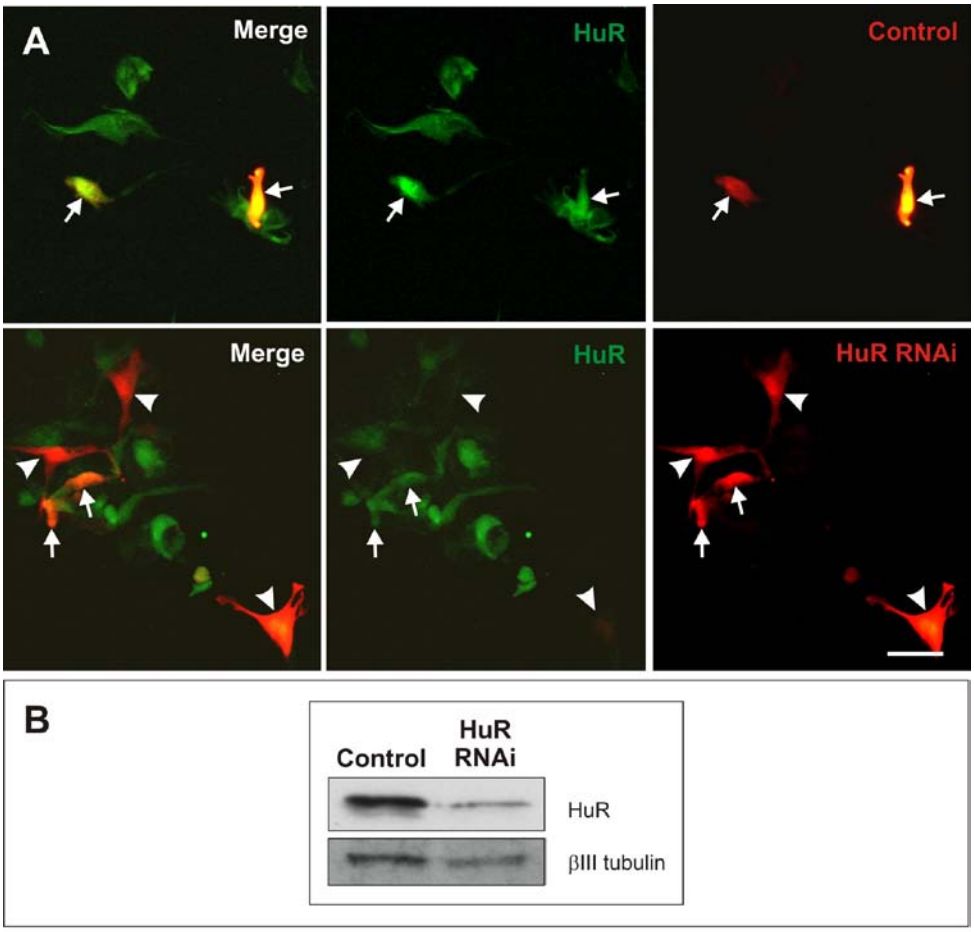

Fig. S5

Figure S6. Elavl1/HuR stabilizes Dll1-3'UTR-containing transcripts in mitotically-synchronized neuroepithelial cells. ED cells were co-transfected with RFP and either *Elavl1* RNAi (HuR RNAi) or a control (Control) construct. These cells were synchronized in M for 24 h, and then treated for either 0 h or 4 h with actinomycin D to block transcription. Those cells expressing high levels of RFP (i.e. with high levels of *Elavl1*-specific RNAi) were subsequently isolated by FACS and the levels of *Egfp* mRNA (normalized to 18S rRNA) were analyzed by quantitative PCR. The results represent the mean ± s.e.m. (n=3). Values from both control and HuR RNAi at 0 h were normalized to 1. *p<0.05 (n=3; Student's *t*-test).

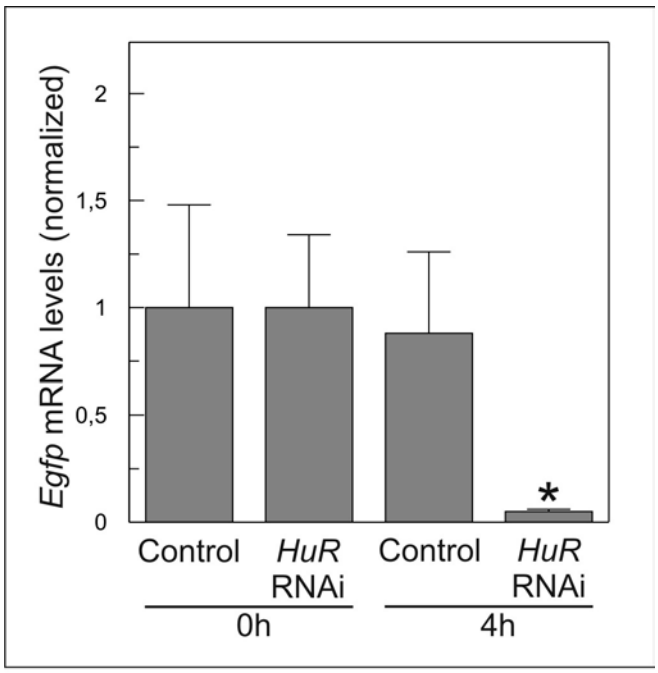

Fig. S6

Table S1. Oligonucleotides used in this study

Name	Accession	Position	Sequence
Notch1 up	NM_008714	7,676-7,695	CTCGCGGCCGCACAGAGATGTGGGATGCAGG
Notch1 down	NM_008714	9,146-9,169	CTGCTTAAGGTAAACTACACTTTATTTTATACA
Dll1 up1	NM_007865	2,517-2,536	CTCGCGGCCGCGATGGAAGCGATGTGGCAAA
Dll1 down1	NM_007865	3,167-3,191	CTGCTTAAGGTAGTTCATTATATTTATTTTGGAA
Dll1 up2	NM_007865	1,893-1,913	GGTACTGCGAGGACAATGTGG
Dll1 down2	NM_007865	2,386-2,408	CCCCAATGATGCTAACAGAAACG
Dll1 forward	NM_007865	1,763-1,783	TTGGGCTTCTCTGGCTTCAAC
Dll1 reverse	NM_007865	1,824-1,845	TCCACACACTTGGCACCGTTAG
Elavl1/1 sense	NM_010485	265-283	GACTGCAGGGATGACATTGTTCAAGAGACAATGTCATCCCTGCA GTCTTTTTT
Elavl1/1 antisense	NM_010485	265-283	AATTAAAAAAGACTGCAGGGATGACATTGTCTCTTGAACAATGT CATCCCTGCAGTCGGCC
Elavl1/2 sense	NM_010485	1,130-1,148	TGGCCATAGCAAGTCTGAATTCAAGAGATTCAGACTTGCTATGG CCATTTTTT
Elavl1/2 antisense	NM_010485	1,130-1,148	AATTAAAAAATGGCCATAGCAAGTCTGAATCTCTTGAATTCAGA CTTGCTATGGCCAGGCC
Elavl1 up	NM_010485	820-841	TCGCAGCTGTACCACTCGCCTG
Elavl1 down	NM_010485	1,005-1,025	CCAAACATCTGCCAGAGGATC
Elavl1 forward	NM_010485	249-267	AGACCACATGGCGGAAGAC
Elavl1 reverse	NM_010485	397-416	CCCAAGCTGTGTCCTGCTAC
Elavl2 up	NM_207685	806-826	AAGCGGATTGAGGCAGAAGAA
Elavl2 down	NM_207685	1,256-1,276	GGCCGCCTCATCATAGTTTGT
Elavl3 up	NM_010487	790-810	TCCGGGATGCCAACCTGTATG
Elavl3 down	NM_010487	1,196-1,216	ATGGCGATTGGGGAGAACCTG
Elavl4 up	NM_010488	936-955	AATGGCCAGAAGCCCAGCGG

Elavl4 down	NM_010488	1,149-1,168	GGGGGACAAGCAGAAGGGGG
Ccnb1 up	NM_172301	465-487	GGAAACATCTGGATGTGCGCCTG
Ccnb1 down	NM_172301	858-879	GTCACCTATTTCTGGAGGGTAC
Ccnb1 forward	NM_172301	1,083-1,102	GGTGCATTTTGCTCCTTCTC
Ccnb1 reverse	NM_172301	1,153-1,172	TGCAGAGTTGGTGTCCATTC
18S rRNA up	X00686	1,577-1,596	GTAACCCGTTGAACCCCATT
18S rRNA down	X00686	1,708-1,727	CCATCCAATCGGTAGTAGCG
18S forward	X00686	1,577-1,596	GTAACCCGTTGAACCCCATT
18S reverse	X00686	1,708-1,727	CCATCCAATCGGTAGTAGCG
Egfp up	U55762	1,305-1,324	CAAAGACCCCAACGAGAAGC
Egfp down	U55762	1,376-1,395	CTTGTACAGCTCGTCCATGC
Egfp forward	U55762	743-762	ACGTAAACGGCCACAAGTTC
Egfp reverse	U55762	801-820	TGAACTTCAGGGTCAGCTTG