di Penta et al., http://www.jcb.org/cgi/content/full/jcb.200807033/DC1

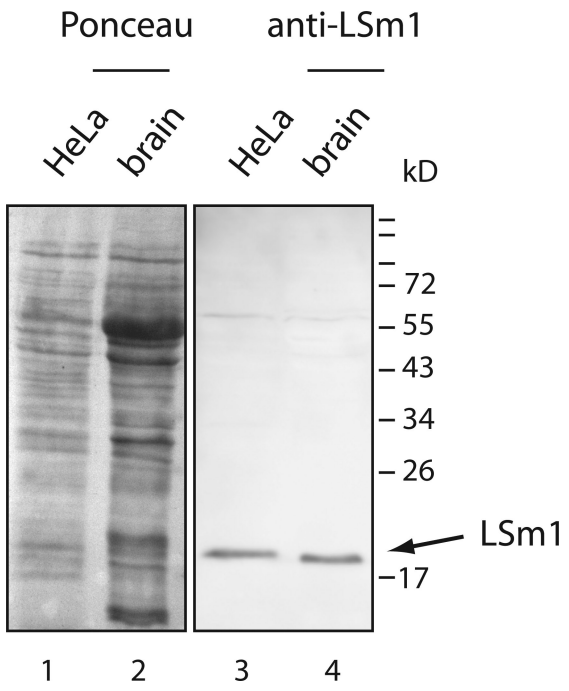

Figure S1. **Specificity of the affinity-purified α-LSm1 antibodies.** The antibodies recognize with great selectivity a single band with an apparent size of 18 kD, which is in good agreement with the theoretical molecular mass of LSm1 (15 kD). Shown is a Western blot of total protein from HeLa cells and rat brain (extracted with RIPA buffer), stained with Ponceau red for total protein (lanes 1 and 2), and then immunostained with affinity-purified α-LSm1 antibodies (lanes 3 and 4). The migration of the molecular mass standards is indicated on the right.

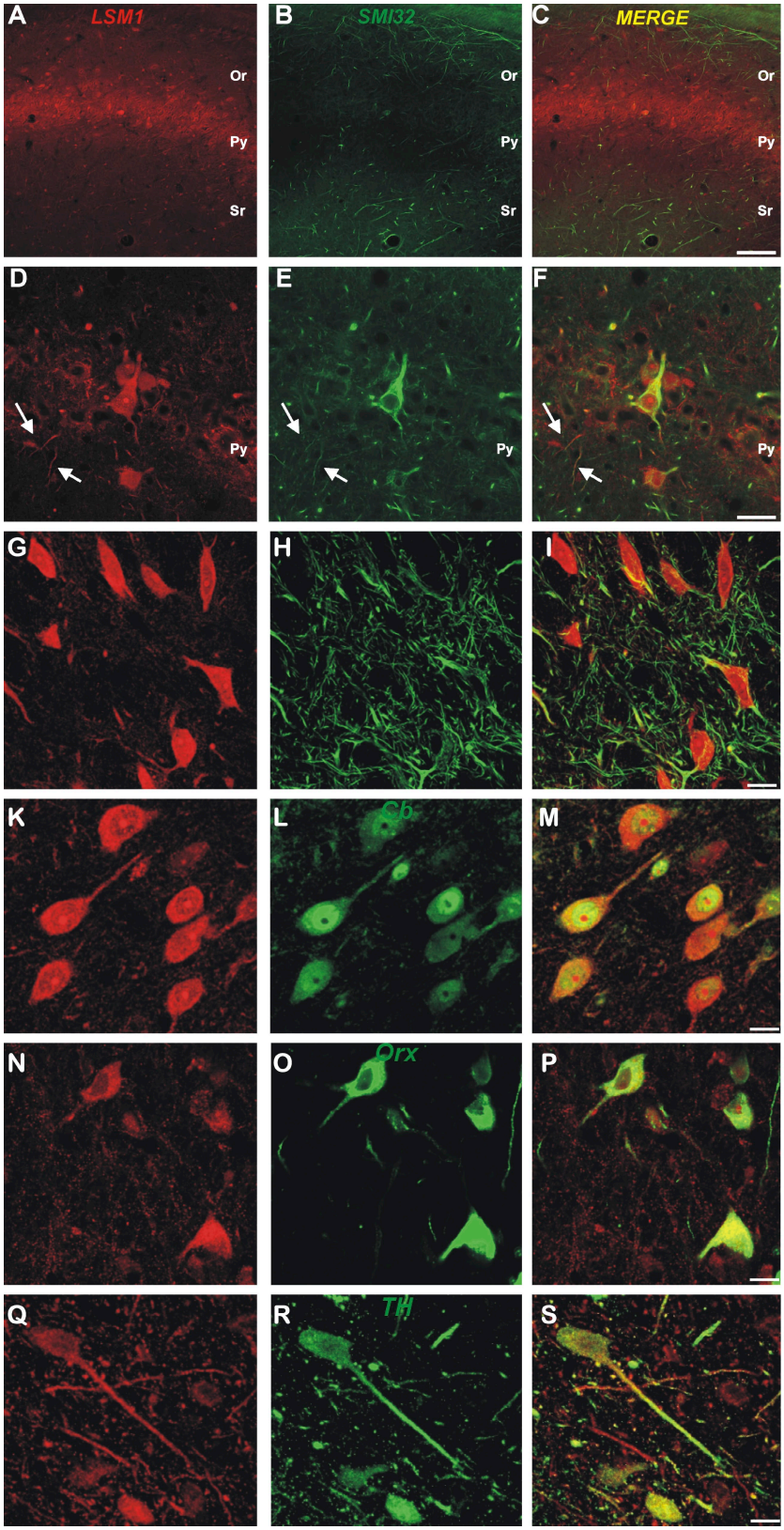

Figure S2. **LSm1 staining in several central nervous system regions.** LSm1-positive neuronal processes can be observed in various cell types with different morphological features in different brain regions. Brain sections were double stained with anti-LSm1 and anti-neurofilament SMI32 (SMI32; A–I), anti-calbindin (Cb; K–M), anti-orexin (Orx; N–P), or anti-tyrosine hydroxylase (TH; Q–S) antibodies. (hippocampus) Low (A–C) and high (D–F) magnification within the pyramidal cell layer. Arrows point to LSm1-positive neuronal processes. (thalamus) Ventrolateral (G–I) and reticular (K–M) thalamic nuclei. LSm1 is present in cell bodies and neuronal processes. (hypothalamus) Perifornical area (N–P). LSm1 is present in neurons and in proximal processes. (substantia nigra) pars compacta (Q–S). LSm1 can be observed in neurons and in proximal and distal processes. Or, stratum oriens; Py, pyramidal cell layer; Sr, stratum radiatum. Bars: (A–C) 200 µm; (D–F) 30 µm; (G–S)15 µm.

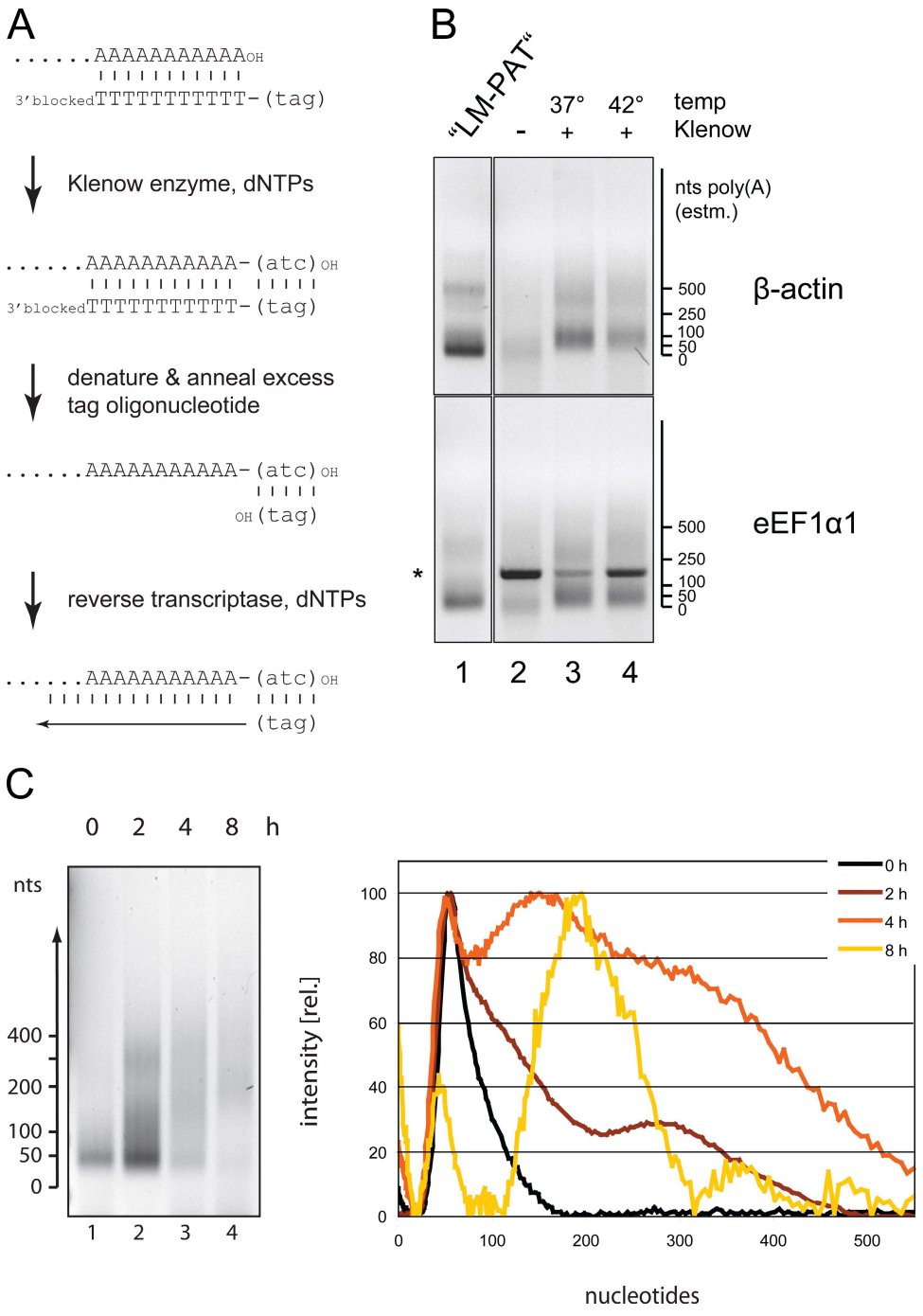

Figure S3. **The tag-addition poly(A) test (TA-PAT).** (A) Schematic description of the method. The method is based on the DNA oligonucleotide-directed extension of an RNA by the Klenow fragment of *Escherichia coli* DNA polymerase I (Sawa, H., and Y. Shimura. 1991. *Nucleic Acids Res.* 19:3953–3958). In brief, the poly(A) ends are elongated by incubation of a T12 tag oligonucleotide (1 pmol in a reaction volume of 5 µl) in the presence of dNTPs and Klenow enzyme (1 U; Roche). It is important that the 3′ OH of the oligonucleotide is blocked and cannot function as primer in the subsequent reverse transcription; in our case, we used a 3′ aminolinker modification. In the second step, the RNA is hybridized with a DNA primer whose sequence is identical to the specific sequence of the first oligonucleotide. To this end, the first reaction is diluted to 50 µl with reverse transcription buffer containing a 10-pmol tag oligonucleotide and dNTPs, and heating to 90°C for 5 min. After cooling to 42°C, reverse transcription is started by the addition of 250 U of superscript III reverse transcription (Invitrogen). (B) Comparison with the ligase-mediated poly(A) test (LM-PAT; Sallés, F.J., W.G. Richards, and S. Strickland. 1999. *Methods.* 17:38–45.). All reaction were performed on 1 µg of the same preparation of rat total RNA; gene-specific primers against actin (top half) and eEF1α (bottom half) were used. As can be seen in lane 1, both mRNAs revealed by LM-PAT show a bimodal distribution with the upper, heterogeneous part corresponding to poly(A) lengths of >250 nts (estimated from the molecular mass markers), whereas the lower part has a prominent, net band corresponding to a very short poly(A) length and a smear that extends to ~100–150 adenosines. When using less starting material, the apparently deadenylated band becomes ever more prominent to the point that the heterogeneous bands indicating poly(A) length can no longer be seen (not depicted). When using the TA-PAT, instead, this lower, sharp band is not observed. Instead, only two homogeneous bands corresponding to poly(A) lengths to 50 to 100 and >250 nts are observed. The temperature of the oligonucleotide annealing/extension reaction was optimized to permit sliding of the oligonucleotide along the poly(A) tail until it reaches the end (lanes 3 and 4): 37°C was chosen for the subsequent experiments. Importantly, the same result was also obtained with much less material (the reactions for the input lanes in Fig. 5 B were performed with 0.1 µg of total RNA). The identity of all bands was confirmed by DNA sequencing; we obtained only one obvious artifact in the eEF1 reaction that can be distinguished by the sharpness of the band and by the fact that it is also observed in the absence of the tag addition (lane 1, asterisk). (C) Validation of the method. *Xenopus laevis* oocytes (stage VI; gift of S. Debaveye, Katholieke Universiteit, Leuven, Belgium) were matured by treatment with progesterone for 0, 2, 4, or 8 h. Total RNA was isolated by acidic phenol extraction, recovered by alcohol precipitation, and further purified by LiCl precipitation. 500 ng of total RNA (equivalent to less than one fourth of an oocyte) was used in each TA-PAT reaction. Cyclin B1, which is polyadenylated early after stimulation, was analyzed (Charlesworth, A., L.L. Cox, and A.M. MacNicol. 2004. *J. Biol. Chem.* 279:17650–17659). The left panel shows the PCR products; the poly(A) length as deduced from the migration of marker DNA is indicated on the left. The right panel shows a graphic representation of the gel image. The intensities were arbitrarily normalized to the strongest signal in each lane and plotted against the estimated poly(A) length. In accordance with previous observations (Charlesworth et al., 2004), cyclin B1 mRNA in resting oocytes has homogenous poly(A) tails of ~50 nts in length (lane 1). Upon stimulation, PCR products representing longer poly(A) tails appear after 2 h (lane 2) and become the prominent signal after 8 h (lane 4).

Table S1. **DNA oligonucleotides used in the study**

Gene targeted	Sequence, 5′ to 3′	Remarks
β-Actin	ACACGGCATTGTCACCAACT CATCACAATGCCAGTGGTAC	exon 3 exon 4
CaMKII	TGAAGGAATCCTCTGAGAGC TGTTCCGGGACCACAGGTTT	exon 8/9 exon 12/13
MAP1B	GCTGTCCTGGATGCCTTGT GGCAGGTCCTTGTTCAGTG	exon 6 exon 7
LSm8 rat for	GACGTCTGCCTTGGAGAACT CAAGTCGAGTGCAGAATCTG	exon 1 exon 4
Eef1a1	CTGATTGTTGCTGCTGGTGT CCATTCCAACCAGAAATTGG	exon 1 exon 1
Itpr1	AACCACGTGCTTCATTTGTG GTGGACTCCAGCTTCTCCTG	exon 59 exon 61
Pcp2	CGTGAGGACTATGGCAGGTT GGTTGAGGGCTGAGTGTCC	exon 1/2 exon 4
Arc	CCTCTCAGGGTGAGCTGAAG CAGGCAGCTTCAAGAGAGGA	exon 1 exon 3
TA-PAT, template	TCCGATAAATAACGCGCCCAATTTTTTTTTTTT	C6-aminolink at the 3′ end
TA-PAT, primer	TCCGATAAATAACGCGCCCAA	
xCycB1	GTGGCATTCCAATTGTGTATTGTT	Charlesworth, A., L.L. Cox, and A.M. MacNicol. 2004. *J. Biol. Chem.* 279:17650–17659.

The first column shows the targeted gene. TA-PAT, template, is the oligonucleotide used in the Klenow fill-in reaction of the PAT procedure and TA-PAT, primer, is the nonspecific primer of the subsequent PCR. The second column lists the sequence of the oligonucleotides. The last column lists, in the case of the gene-specific primers, the exons to which the primers anneal. Numbering is according to the Gene Table function of the National Center for Biotechnology Information Gene database. Where two numbers are given, the primer anneals over an exon–exon boundary.