Supplementary Figures

Figure S1: *In vitro* neural differentiation of mouse embryonic stem cells; A. Mouse embryonic stem (ES) cells uniformly express Oct4 (green), ES cell-derived neural progenitors (NP) uniformly express nestin (red), while ES cell-derived neurons and glial cells (N/P), express Tau (green), Gfap (green) or Osp (yellow) as revealed by immunocytochemistry. Nuclei stained with DAPI. Scale bar: 20µm. B. Relative mRNA expression levels during neural differentiation in the mouse ES cells *in vitro*. Neural differentiation is accompanied by loss of *Nanog* and *Oct4* expression and by up-regulation of the expression of the neural markers *Nestin* and *Sox1*, the neuronal marker *Tau*, the astrocytic marker *Gfap* and the oligodendrocytic marker *Osp*. Data are presented as mean ± SEM; n=3; * p<0.05; ** p<0.01 significantly different from ES cells by Student t-test.

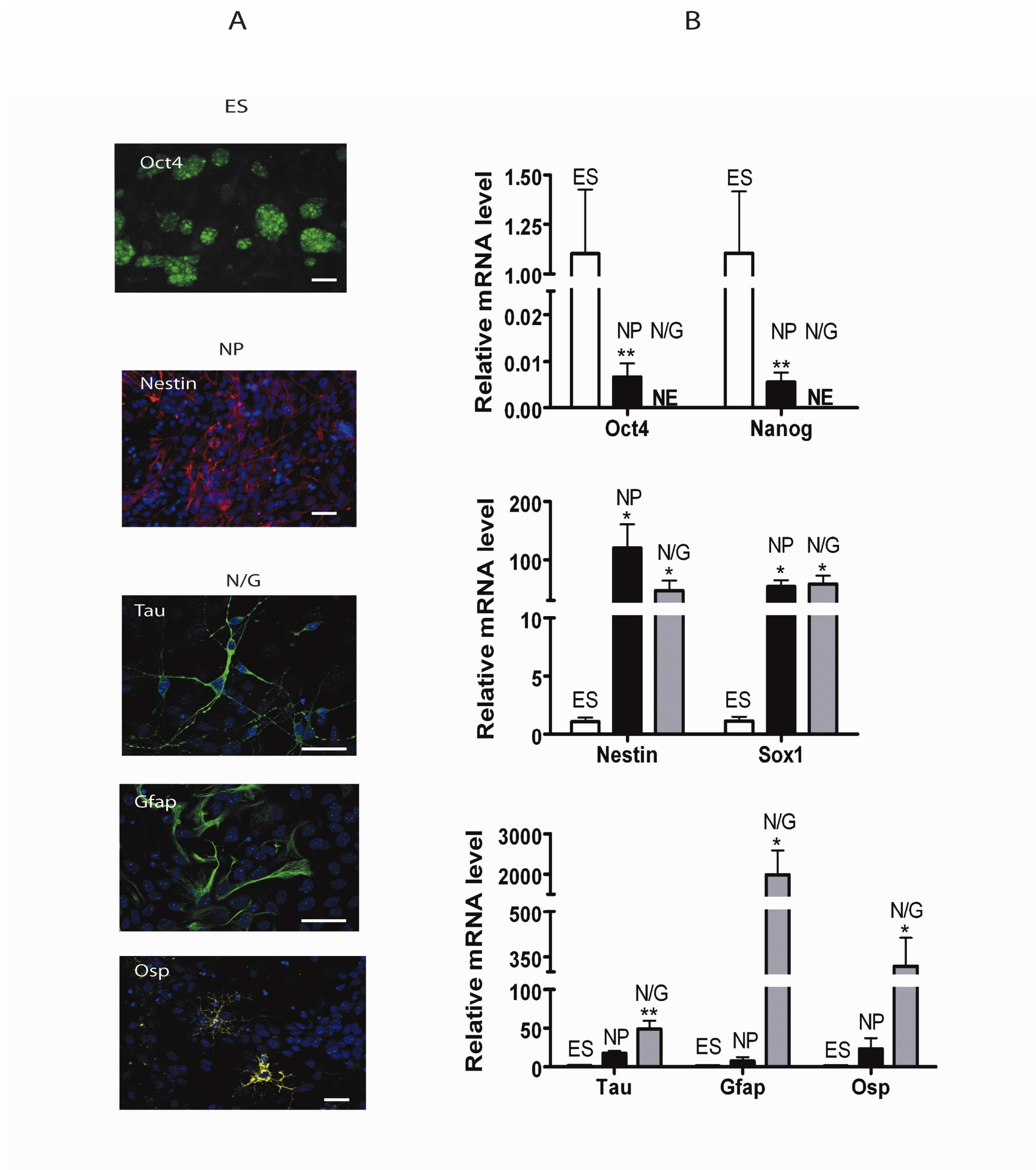


Figure S2: Detection of processing events of intergenic ncRNAs and microRNAs in the ES, NP and N/G libraries by the APART pipeline. Results are represented by employing the UCSC genome browser. Reads shown next to each processing event are not normalized.

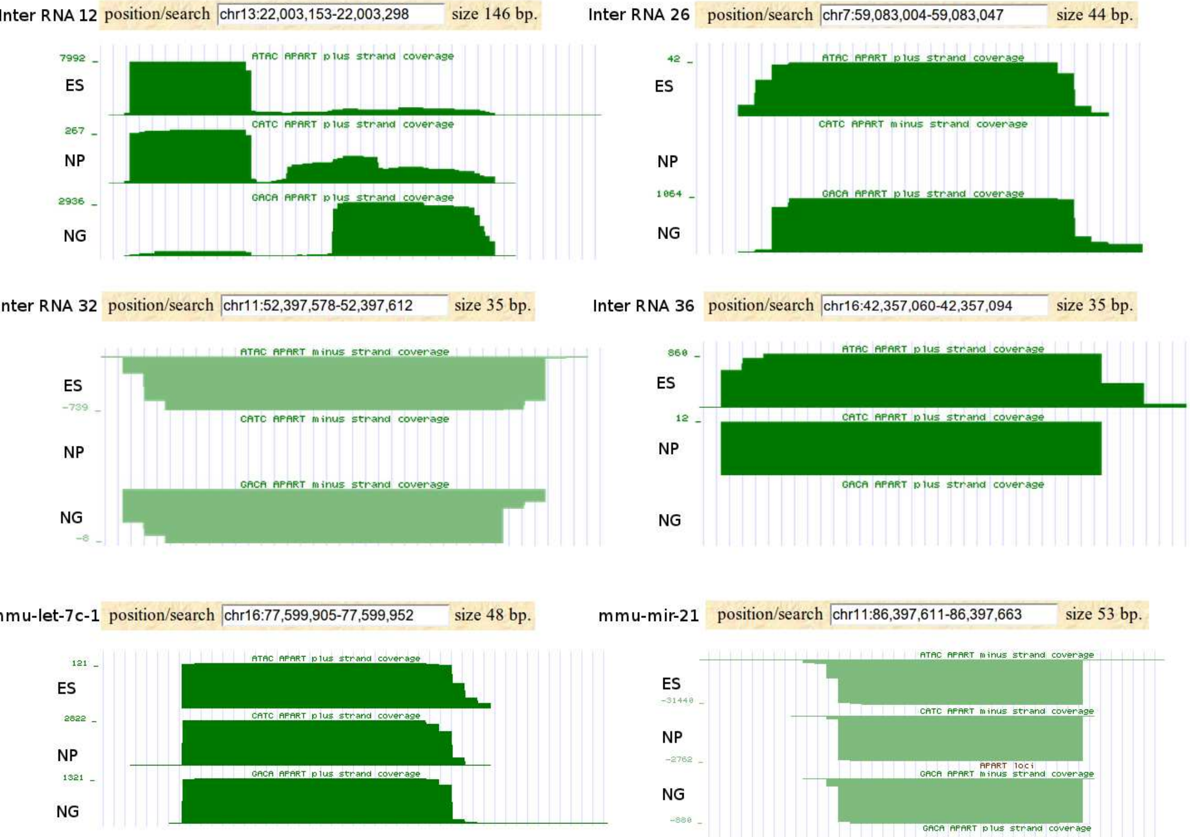


Figure S3: Northern blot analysis from nuclear RNP extracts showing differential expression of SNORD115 in ES cells and differentiated cells. As a loading control, 5.8S RNA was used.

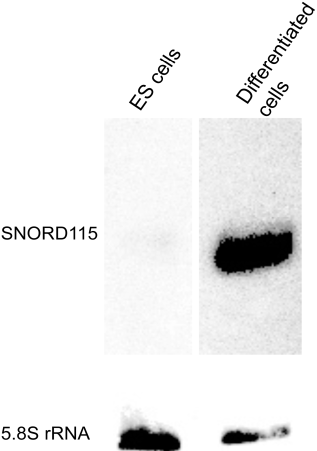
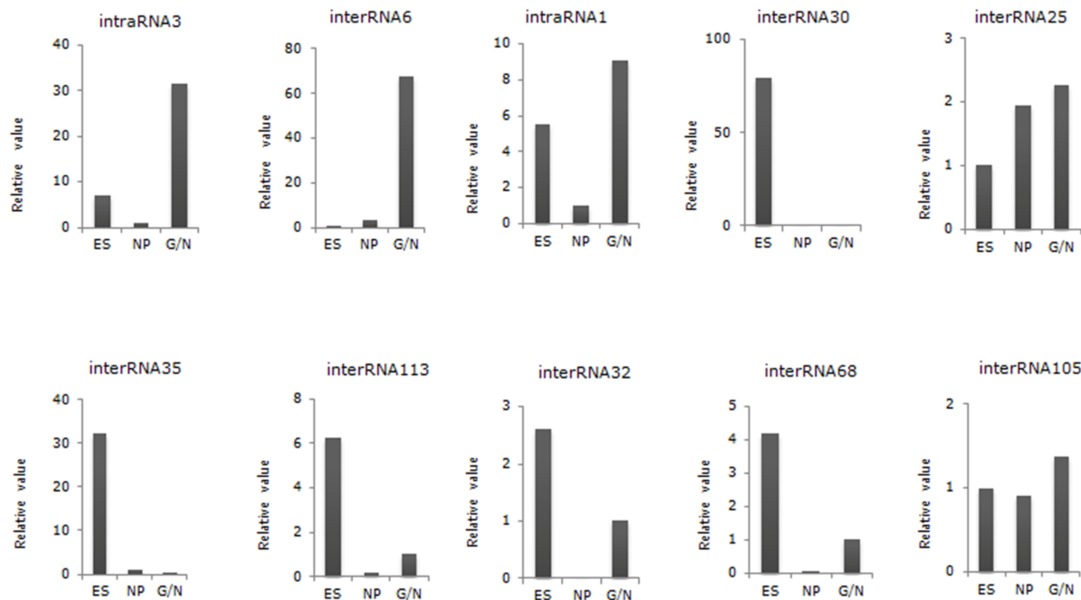


Figure S4: Verification of differential expression analysis of selected intra- and interRNAs by real-time PCR.



Supplementary methods

Immunocytochemistry

Adherent cells on glass coverslips were fixed with 4% buffered paraformaldehyde in phosphate-buffered saline (PBS; 0.01 M, pH 7.4), for 20 minutes at RT and washed twice with PBS. Permeabilization was carried out using 0.3% Triton-X-100 in PBS (PBST) for 1 h at RT. After blocking with serum (10% goat normal serum, 1% BSA in PBST), cells were incubated overnight at 4℃ with primary antibodies diluted in 1% BSA in PBST: Oct4, nestin (1:200, mouse IgG SantaCruz), Tau (1:200, mouse IgG SantaCruz or 1:1000 rabbit IgG DAKO), oligodendrocyte specific protein (OSP, 1:200 rabbit IgG, Abcam), and glial fibrillary acidic protein (GFAP, 1:1000 rabbit IgG DAKO). After several rinses in PBST, cells were incubated for 1 h with AlexaFluor secondary antibodies (Alexa 488-conjugated goat anti-mouse and Alexa 555-conjugated goat anti-rabbit, 1:2000, Invitrogen) and washed 3 times in PBS. The coverslips were incubated for 2 min with 4', 6-diamidino-2-phenylindole (DAPI, 300 nM in PBS) for nuclear staining, rinsed in PBS and deionized water and mounted on slides with Mowiol (Sigma). Negative controls, without primary antibody were performed in all experiments to monitor the non-specific staining. Pictures were

taken with an ApoTome Imaging System based on Axio Observer Z1 (Zeiss) using AxioVision software.

Quantitative Real-Time PCR

Messenger RNA was isolated from E14 mouse embryonic stem cells and their derived progenies using Dynabeads® Oligo (dT) 25 (Invitrogen Corporation, Paisley, UK) following the manufacturer's protocol. The eluted mRNA was immediately used for cDNA synthesis according to High-Capacity cDNA Reverse Transcription Kit protocol (Applied Biosystems, Warrington, UK). The levels of cDNA for the mouse cells were assessed by quantitative real-time PCR using Fast SYBR® Green Master Mix (Applied Biosystems, Life Technologies Corporation, Carlsbad, California, USA). Standard curves and melting curves were determined for each set of primers to confirm that a single amplicon was generated. All results from three technical replicates were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expressed as ΔCt values (low ΔCt levels indicate high expression). Relative expression ratios were calculated by the ΔΔCt method (78).
For statistical analyses, the Statview (version 5.0.1) software was used (SAS Institute, Carry NC). Three independent biological samples for each time point contributed to the data set. Data are presented as mean ± standard error of the mean (SEM). Student t-test was applied to compare between two groups. Differences were considered significant when $p<0.05$.

Preparation of dissociated hippocampal neurons

Dissociated hippocampal cultures were prepared from P0 C57Bl6 mice as previously described (79) with modifications. Hippocampi were dissected out in ice-cold complete HBSS, dissociated by incubation in 0.25% trypsin/EDTA for 5 min at 37℃, and triturated with a fire-polished Pasteur p ipette (3 rounds). Hippocampal cells were plated at high density (1×10^6 cells/dish) on 35 mm dishes coated with poly- L-ornithine (Sigma) and laminin (Sigma) in Neurobasal A medium (Life Technologies) containing 2% (vol/vol) B27, 1X GlutaMax, 100 U/mL penicillin, 100 µg/mL streptomycin. Medium was replaced 2 h later; half-change was made after 4 days in culture. At DIV7 hippocampal neurons were lysed in Trizol for RNA extraction.

Preparation of glial culture

Glial cells were prepared from the brains of neonatal (3 to 5-day-old) C57Bl6 mice as previously described (80) with modifications. Briefly, cortices of 2-3 brains were dissected in complete HBSS – 1X HBSS (Life Technologies), 2.5 mM Hepes (pH 7.4, Life Technologies), 30 mM D-glucose (Life Technologies), 1 mM $CaCl_2$ (Sigma), 1 mM $MgSO_4$ (Sigma), 4 mM $NaHCO_3$ (Sigma); minced with scissors; and incubated in 0.25% trypsin/EDTA (Life Technologies) for 15 min at 37℃. After trituration by a fire-polished Pasteur pipette (3 X 3 triturations) the cell suspension was passed through a 40 µm cell strainer and centrifuged at 400 × *g*. The pellet was re-suspended in culture medium (1X DMEM, containing 4,500 mg/mL glucose, GlutaMAX-I, 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin – all reagents from Life Technologies). The cells were plated on 60 mm dishes and cultured at 37℃ in a humidified a tmosphere containing 5% $C0_2$. After 24 hours, plates were washed to remove non-adherent cells and debri. Medium was changed every 3-4 days. Cultures were passaged twice (at 10 day interval) and used in the experiments after the second passage.

Scoring function

To score expression values, following parameters were employed:

1. Mean fold change:

   For ncRNAs, which are present in all three stages (i.e. cDNA libraries), the value (modulus) of each fold-change between stages (stage 1 versus stage 2, stage 2 versus stage 3 and stage 1 versus stage 3; N = 3) was added up and divided by N.

2. Mean difference in expression values:

   The differences of expression values (modulus) were added up and divided by the number of comparisons.

The final score results from multiplication of the mean fold change and the mean difference in expression values, both normalized to one.

Real-time PCR for verification of bioinformatically predicted candidates

cDNA deriving from the three RNP libraries was used for verification of the bioinformatically predicted ncRNA expression. Real-time PCR was performed at the same conditions as described in the materials and methods section, using the 3' libPCR primer as reverse. Normalization was performed with U5. Following primers were used:

mmu-intraRNA1: GAACGAGATTCCCACTGTCCCTAC

mmu-interRNA6: GAACGTGAGCTGGGTTTAGACCGTC

mmu-intraRNA3: GAAACCACAGCCAAGGGAACG

mmu-interRNA25: GGAGAAGGGTTCCATGTGAACAG

The primers for the remaining inter- and intraRNAs depicted in Figure S4 are described in the materials and methods.