SUPPLEMENTARY DATA

Supplementary Figures.

Supplementary Figure 1. Knockdown of LANP promotes neurite outgrowth in N2A cells.

A-D. N2A cells were transfected with control siRNA (A and C) or siRNA targeting LANP (B and D) in the presence of cAMP. 48h post transfection, the cells were fixed and stained with anti-tubulin (A and B) and anti-LANP (C and D) antibodies.

E. Quantitation of number of cells displaying neurites longer than two cell body length. Mock represents untransfected cells. Scale bar= 20μ m. Cells were counted in 3 sets of high power fields representing triplicate transfections (typically 6 fields or more were counted with total number of cells >75 per set). Error bars represent SEM; p<0.005.

F. N2A cells were transfected with siRNA targeting LANP and control siRNA. 48h post transfection, western blot analysis was performed with anti-LANP antibody. Actin was used as a loading control. Mock represents untransfected cells.

Supplementary Figure 2. Knockdown of LANP by RNA interference results in upregulation of NF-L transcripts 24h post-transfection.

A. PC12 cells transfected with siRNA targeting LANP show reduced expression at the RNA level 24h post transfection as demonstrated by RT-PCR. In these experiments, GAPDH message was measured as an internal control.

B. PC12 cells transfected with a pool of siRNA targeting LANP and control siRNA show reduced level of LANP protein 24h post transfection by western blot analysis. Actin was used as a loading control.

C. Semi-quantitative analysis from triplicate samples depicts 66% knockdown of LANP protein levels. LANP protein levels are relative to actin and normalized to protein levels of LANP upon transfection with control siRNA.

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D. RNA was isolated from PC12 cells 24 hours after transfection with siRNA targeting LANP and control siRNA. DNAse treated RNA was subjected to quantitative-RT-PCR analysis employing primers to amplify NF-L transcripts. Ct value of each sample is normalized to GAPDH and fold change is relative to control transfected PC12 cells.

Supplementary Figure 3. Knockdown of LANP does not alter histone acetylation status at the control β -globin promoter. PC12 cells were transfected with siRNA targeting LANP or control siRNA. 48h post transfection, cells were processed for chromatin immunoprecipitation with anti-acetyl H3 (H3 ac), anti-H3 total antibody or IgG (serving as a negative control). PCR was performed to amplify the promoter region of β -globin gene (serving as a control). Pre-immunoprecipitated lysate served as input.

Supplementary Figure 4. Depletion of LANP does not alter histone acetylation status at the β-globin promoter. Postnatal day 2 mice were sacrificed and brains were processed for chromatin immunoprecipitation with anti-acetyl H3, anti-H3 total or IgG (serving as control) antibody. PCR was performed to amplify the promoter region of β-globin (serving as a control). Pre-immunoprecipitated lysate served as input.

Supplementary Table 1. The list of differentially expressed genes in control and siRNA transfected PC12 cells as depicted in Figure 3. Signal intensity, fold-change, p value and gene symbol annotation with accession numbers are displayed. Genes are grouped according to their major biological function.







F.

with neurites









Supplementary Table 1

| | Group | Exp Group | Raw Est Fold | | | |
|---|---------|-----------|-----------------|---------|----------------|----------------|
| Gene_Descriptor | Signal | Signal | Change | p-value | Gene_Symbol | SeqDerivedFrom |
| acidic (leucine-rich) nuclear phosphoprotein 32 family, member A | 1151.4 | 203.0 | -5.67 | 0.0152 | Anp32a | NM_012903 |
| acidic (leucine-rich) nuclear phosphoprotein 32 family, member E (predicted) | 2473.0 | 2057.1 | -1.20 | 0.0012 | Anp32e | AI008642 |
| acidic nuclear phosphoprotein 32 family, member B | 4342.8 | 4068.0 | -1.07 | 0.4954 | Anp32b | NM_131911 |
| | | | | | | |
| Differentiation | | | | | | |
| epidermal growth factor | 60.9 | 304.1 | 5.00 | 0.0021 | Egf | NM_012842 |
| vascular endothelial growth factor C | 15.0 | 65.5 | 4.37 | 0.0097 | Vegfc | NM_053653 |
| brain derived neurotrophic factor | 77.0 | 248.2 | 3.23 | 0.0000 | Bdnf | X67108 |
| Synapsin I | 266.8 | 490.6 | 1.84 | 0.0016 | Syn1 | BG378858 |
| neurohilament 3, medium | 3750.4 | 6524.0 | 1.74 | 0.0001 | Nef3 | NM_017029 |
| growth associated protein 43 | 413.7 | 617.6 | 1.49 | 0.0088 | Gap43 | NM_017195 |
| neurohlament, light polypeptide | 2595.6 | 3601.0 | 1.39 | 0.0006 | RGD:621458 | BF394545 |
| neurohlament, heavy polypeptide | 1912.9 | 2457.2 | 1.29 | 0.0232 | Neth | AF031879 |
| Vascular endothelial growth factor A | 777.0 | 456.0 | -1.70 | 0.0302 | Vegt | AF080594 |
| neural precursor cell expressed, developmentally down-regulated gene 3 (predicted) | 708.8 | 368.5 | -1.92 | 0.0012 | Nedda | BF555368 |
| colony stimulating factor I (macrophage) | 189.9 | 95.0 | -2.00 | 0.0059 | LSTI | AW 535553 |
| Keratinocyte differentiation associated protein | 143.9 | /1.1 | -2.02 | 0.0036 | Kittap | B1282368 |
| noroblast growth factor 7 | 1290.5 | 611.3 | -2.11 | 0.0276 | Fgr/ | NM_022182 |
| Insulin-like growth factor binding protein 5 | 3285.7 | 1101.8 | -2.98 | 0.0309 | cqaigi | BF333783 |
| Cell cuole/Cancer | | | | | | |
| cell cycle/Cancel | 26.7 | 120.2 | 2.52 | 0.0022 | | PC 200026 |
| similar to downlegalated in renar cell carcinoma (predicted) | 160.0 | 128.3 | 3.52 | 0.0032 | 1.00.406500 | DM300020 |
| Tumor necrosis factor recentor superfamily, member 26 (predicted) | 205.6 | 933.7 | 2.00 | 0.0003 | 200430203 | PE099217 |
| PAP15 member PAS encodered family, member 20 (predicted) | 005.0 | 1007.4 | 2.20 | 0.0010 | P-b15 | DE030317 |
| high mohilitu aroun AT-book 1 | 755 6 | 1576.0 | 2.13 | 0.0000 | Hmgal | BG378885 |
| cuclin D1 | 851.0 | 1814 9 | 2.03 | 0.0002 | Cend1 | BI295861 |
| Death-associated protein | 111 2 | 218.2 | 1 06 | 0.0016 | Dan | BE110708 |
| cyclin D1 | 1004.2 | 1689.6 | 1.60 | 0.00186 | Cond1 | AW143798 |
| Cyclin G2 (predicted) | 3004.2 | 181.9 | -1.66 | 0.0186 | Cong2 | AI408309 |
| cyclin E | 713.4 | 423.4 | -1.00 | 0.0100 | Conge Conge | AW913890 |
| cvclin L2 (predicted) | 329.2 | 169.0 | -1.00 | 0.0070 | Conl2 | BE098333 |
| CDC23 (cell division cycle 23, yeast, homolon) (predicted) | 359.0 | 138.6 | -1.55 | 0.0625 | Cdc23 | AI013729 |
| en arrent syste ze, year, nonology (productor) | 555.8 | . 30.0 | -2.00 | 0.3023 | | |
| Neurodegeneration | | | | | | |
| homer homolog 1 (Drosophila) | 32.8 | 73.1 | 2.23 | 0.0077 | Homer1 | AF030088 |
| hane morphogenetic protein 2 | 1276.1 | 2773.8 | 217 | 0.0045 | Bmn2 | AA944827 |
| VGE nerve growth factor inducible | 4813.3 | 9470.8 | 1.97 | 0.0025 | Vaf | NM 030997 |
| Huntingtin interactor protein E | 396.3 | 728.3 | 1.84 | 0.0020 | LOC288741 | BM384029 |
| atavin 2 | 280.6 | 497.0 | 1.01 | 0.0040 | 200200111 | AI060139 |
| Purkinie cell protein 4 | 296.8 | 159.6 | -1.86 | 0.0182 | Pcp4 | NM 013002 |
| gamma-aminobuturic acid receptor, subunit beta 3 | 119.0 | 59.2 | -2.01 | 0.0001 | Gabrb3 | NM 017065 |
| gamma-aminobutvric acid (GABA) A recentor, pi | 224.3 | 79.7 | -2.82 | 0.0484 | Gabro | NM 031029 |
| ataxin 3 | 136.6 | 37.6 | -3.64 | 0.0434 | Atxn3 | NM 021702 |
| | | | 0.01 | 0.0101 | | |
| Transcription/Translation | | | | | | |
| Cbp/p300-interacting transactivator | 944.7 | 1923.8 | 2.04 | 0.0012 | Cited2 | AI013390 |
| histone 3, H2ba (predicted) | 414.9 | 232.3 | -1.79 | 0.0364 | Hist3h2ba | AW533007 |
| H3 histone, family 3B | 11898.5 | 6653.1 | -1.79 | 0.0265 | RGD:621095 | AI177503 |
| Histone 1, H4a (predicted) | 4690.4 | 2584.9 | -1.82 | 0.0004 | | AA848821 |
| CCAAT/enhancer binding protein (C/EBP), gamma | 268.2 | 141.4 | -1.90 | 0.0087 | Cebpg | X64403 |
| meningioma expressed antigen 5 (hyaluronidase) | 602.7 | 304.6 | -1.98 | 0.0021 | Mgea5 | NM_131904 |
| histone 2 | 97.2 | 49.1 | -1.98 | 0.0170 | Hist2h2aa | AI235236 |
| general transcription factor II H, polypeptide 1 (predicted) | 284.8 | 132.9 | -2.14 | 0.0032 | Gtf2h1 | BF524412 |
| histone 2a | 533.8 | 243.7 | -2.19 | 0.0178 | RGD:621437 | AA850895 |
| histone 1, H2bh (predicted) | 1382.4 | 614.0 | -2.25 | 0.0181 | Hist1h2bh | BM391206 |
| eukaryotic translation initiation factor 4, gamma 2 (predicted) | 7868.9 | 3194.2 | -2.46 | 0.0308 | Eif4g2 | BG672378 |
| histone 1, H2bl | 37.5 | 10.3 | -3.66 | 0.0001 | Hist1h2bl | NM_022647 |
| | | | | | | |
| Signal transduction | | | | | | |
| microfibrillar associated protein 5 (predicted) | 44.2 | 141.7 | 3.20 | 0.0010 | Mfap5 | BI283094 |
| serine (or cysteine) proteinase inhibitor, clade B, member 2 | 40.4 | 128.3 | 3.17 | 0.0035 | Serpinb2 | NM_021696 |
| similar to MAP kinase phosphatase 4 | 221.5 | 667.8 | 3.02 | 0.0008 | LOC293847 | AI179321 |
| bradykinin receptor b2 | 118.5 | 303.6 | 2.56 | 0.0001 | Bdkrb2 | BF408558 |
| mitogen activated protein kinase kinase kinase 12 | 46.4 | 102.9 | 2.22 | 0.0036 | марзк12 | BI303658 |
| Interieukin Treceptor accessory protein Dis GTDsss asticular system 27 | 98.0 | 202.8 | 2.07 | 0.0019 | httap | DF331314 |
| nnu u i nase activating protein 27 | 221.0 | 445.0 | 2.01 | 0.0037 | Arngap 15 | DE 100015 |
| a protein-coupled receptor 40 | 429.0 | 221.2 | -1.94 | 0.0026 | dpr46 | DI3UU274 |
| aipriorz-matriogiobulin adrenarzia recenter, beta 2 | 628.9 | 412.7 | -2.01 | 0.0000 | Addb2 | NM_012109 |
| aurenergio receptor, peta 5 aurenine nucleotide hinding protein (Claustein), assume 5 automit | 208.8 | 400.0 | -2.11 | 0.0026 | Adio3 | NM_013108 |
| guarina nucleotude binoing protein (di protein), gamma 5 subunit prostanlandia E recentor 2 (subtune EP2) | 264.9 | 123.6 | -2.14 | 0.0048 | ungo Plaar2 | NM_024377 |
| prostagianum E receptor 5 (subtype En 5) quanulate nucleotide binding protoin 2 | 1472.1 | 000.1 | -2.15 | 0.0009 | Figers Sho2 | NM_012704 |
| Ismin B recentor | 404./ | 211.2 | -2.20 | 0.0017 | L br | NM 134453 |
| matrix metallopentidase 2 | 147.0 | 310./ | -2.33 | 0.0036 | Mmp2 | 165656 |
| serine (or custeine) proteinase inhibitor, clade A | 2202.7 | 976 9 | -2.47 | 0.0011 | Seroina6 | 66893495 |
| interleukin 1 recentor, type I | 2303.7 | 0.010 | -2.03 | 0.0101 | l11r1 | NM 013123 |
| and a second of the second s | 200.9 | 00.3 | -4.00 | 0.0121 | | 0.0120 |
| Miscellaneous | | | | | | |
| prostaglandin-endoperoxide synthase 2 | 517 9 | 2866.1 | 5 53 | 0.0000 | Ptas2 | U03389 |
| aquaporin 8 | 648.1 | 2743.5 | 4.23 | 0.0000 | Agp8 | NM 019158 |
| kallikrein 10 | 110.6 | 324.1 | 2.93 | 0.0000 | RGD:1303242 | BI283346 |
| Kruppel-like factor 5 | 47.5 | 135.2 | 2.84 | 0.0036 | Klf5 | BM382886 |
| Potassium voltage-gated channel, subfamily G, member 1 | 61.8 | 166.6 | 2.04 | 0.0010 | Keng1 | BF387447 |
| NOD3-like protein (has Leucine-rich repeats) | 119.2 | 256.1 | 2.15 | 0.0005 | nod3l | BE106776 |
| DnaJ (Hsp40) homolog, subfamily C, member 4 (predicted) | 230.0 | 118.4 | -1.94 | 0.0004 | Dnaic4 | BG380252 |
| SET binding factor 1 (predicted) | 332.7 | 162.6 | -2.05 | 0.0360 | Sbf1 | AW/434982 |
| adrenergic receptor, beta 3 | 208.8 | 99.1 | -2.11 | 0.0026 | Adrb3 | NM 013108 |
| Nuclear pore associated protein | 551.3 | 243.7 | -2.26 | 0.00088 | | BI283822 |
| lamin B receptor | 747 6 | 318.7 | -2.20 | 0.0056 | Lbr | NM 134453 |
| crystallin, gamma E | 369.3 | 146.6 | -2.50 | 8300.0 | Crvge | J00716 |
| calcium channel, voltage-dependent, alpha2/delta subunit 1 | 909.4 | 351.4 | -2.52 | 0.0003 | Cacna2d1 | AF400662 |
| sodium channel, voltage-gated, type III, beta | 3576.8 | 1274.8 | -2.81 | 0.0000 | Scn3b | AA685184 |
| 5-hydroxytryptamine (serotonin) receptor 2C | 58.6 | 18.4 | -3.19 | 2000.0 | Htr2c | U35315 |
| angiotensin II receptor, type 2 | 327.2 | 95.7 | -3.42 | 0.0011 | Agtr2 | BF552873 |
| · · · · · · · · · · · · · · · · · · · | | 4.0 | 27.75 | 0.0008 | Tuk | NM 012077 |

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