Supplementary Data

A Nuclear Function for the Presenilin 1 Neuronal Partner NPRAP/δ-Catenin

Carolina Koutras^{a,b}, Christian B. Lessard^{a,c} and Georges Lévesque^{a,*} ^aDepartment of Psychiatry-Neurosciences, Faculty of Medicine, Laval University and Neuroscience Unit, CHUL, QC, Canada

^bTanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada ^cDepartment of Neurosciences, University of California at San Diego, La Jolla, California, USA

Handling Associate Editor: Stephen Ginsberg

Accepted 12 June 2011

WESTERN BLOT

Protein expression was validated using western blot analysis. Briefly, cells were centrifuged for 5 min at 1,000 × g and resuspended in 500 μ l of STEN buffer (50 mM Tris, pH 7.6, 150 mM NaCl, 2 mM EDTA, 0.2% NP-40 and 0.5% Triton) supplemented with a protease inhibitor cocktail (Complete[®], Roche). After 30 min of incubation with agitation at 4°C, lysates were passed several times through a 25-gauge needle and centrifuged at 12,000 rpm for 10 min (4°C). The protein concentration of the supernatant was determined using Bradford reagent (Biorad). Equal amounts of protein were mixed with Laemmli buffer, boiled at 95°C for 5 min, separated using 10% SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. Membranes were incubated in 5% nonfat milk in a Tris-buffered solution containing 0.1% Tween (TBS-T) for 1 h and subsequently probed with a mouse anti-Xpress tag antibody (Invitrogen). After a series of washes in TBS-T, the membrane was reprobed with a donkey anti-mouse antibody conjugated to horseradish peroxidase (Santa Cruz) for 1 h. Proteins were visualized using an ECL reagent (Perkin Elmer).

^{*}Correspondence to: Georges Lévesque, Neuroscience Unit, CHUL Research Center, 2705 Boulevard Laurier RC9800, G1V 4G2, QC, Canada. Tel.: +1 418 654 2152; Fax: +1 418 654 2753; E-mail: Georges.levesque@crchul.ulaval.ca



Supplementary Figure S1. Related to Table 1. Western blot analysis of NPRAP expression in samples subjected to microarray hybridization. NPRAP expression in cells transfected either with an empty vector or an NPRAP-encoding vector (lanes 1 and 2) was compared to a cell line stably overexpressing NPRAP (lane 3) as a control for NPRAP expression. Cells from lanes 1 and 2 conditions were further subjected to RNA extraction and microarray hybridization. This protein expression verification was performed in all samples subjected to microarray analysis.



Supplementary Figure S2. Results of the β -Gal activity assay using CPRG as a substrate for relative quantification of interaction strengths between PS1 and NPRAP. The y-axis shows the calculated units of β -Gal activity of AH109 yeast cells expressing prey and bait PS1 and NPRAP proteins (x-axis)S.

Supplementary Table S1 Related to Tables 1 and 2. An example of biological and pathological processes regulated by NPRAP target genes

Process	Gene	Role	Reference
Alzheimer's Disease	BCHE	Risk factor	[1]
	MEOX2	Neurovascular dysfunction	[2]
Neurite modulation	SLITRK5	Neurite-modulating activity	[3]
	DOK6	Ret-mediated neurite outgrowth	[4]
Cancer	PCDH10	Tumor suppressor gene	[5]
	CDH11	Prostate cancer metastasis	[6]
	EDNRB	Nasopharyngeal carcinoma Uveal melanoma	[7, 8]
	RNASEL	Prostate tumorigenesis Pancreatic tumorigenesis	[9, 10]
	BCHE	Colorectal carcinoma	[11]
	PTGER4	Colorectal carcinogenesis	[12]
	IF116	Prostate tumorigenesis	[13]
	LCP1	Colorectal cancer metastasis	[14]
	FN1	Ovarian tumorigenesis Breast tumorigenesis	[15, 16]
Connective tissue disorder	COL3A1	Ehlers-Danlos syndrome type 4	[17]

REFERENCES

- McIlroy SP, Crawford VL, Dynan KB, McGleenon BM, Vahidassr JT, Passmore MD, Lawson AP (2000) Butyrylcholinesterase K variant is genetically associated with late onset Alzheimer's disease in Northern Ireland. *J Med Genet* 37, 182-185.
- [2] Wu Z, Guo H, Chow N, Sallstrom J, Bell RD, Deane R, Brooks AI, Kanagala S, Rubio A, Sagare A, Liu D, Li F, Armstrong D, Gasiewicz T, Zidovetzki R, Song X, Hofman F, Zlokovic BV (2005) Role of the MEOX2 homeobox gene in neurovascular dysfunction in Alzheimer's disease. *Nat Med* **11**, 959-965.
- [3] Shmelkov SV, Hormigo A, Jing D, Proenca CC, Bath KG, Milde T, Shmelkov E, Kushner JS, Baljevic M, Dincheva I, Murphy AJ, Valenzuela DM, Gale NW, Yancopoulos GD, Ninan I, Lee FS, Rafii S (2010) Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsivelike behaviors in mice. *Nat Med* 16, 598-602.
- [4] Crowder RJ, Enomoto H, Yang M, Johnson EM, Jr, Milbrandt J (2004) Dok-6, a Novel p62 Dok family member, promotes Ret-mediated neurite outgrowth. *J Biol Chem* 279, 42072-42081.
- [5] Ying J, Li H, Seng TJ, Langford C, Srivastava G, Tsao SW, Putti T, Murray P, Chan ATC, Tao Q (2006) Functional epigenetics identifies a protocadherin PCDH10 as a candidate tumor suppressor for nasopharyngeal, esophageal and multiple other carcinomas with frequent methylation. *Oncogene* 25, 1070-1080.
- [6] Bussemakers MJ, Van Bokhoven A, Tomita K, Jansen CF, Schalken JA (2000) Complex cadherin expression in human prostate cancer cells. *Int J Cancer* 85, 446-450.
- [7] Smith SL, Damato BE, Scholes AGM, Nunn J, Field JK, Heighway J (2002) Decreased endothelin receptor B expression in large primary uveal melanomas is associated with early clinical metastasis and short survival. *Br J Cancer* 87, 1308-1313.

- [8] Lo K-W, Tsang Y-S, Kwong J, To K-F, Teo PML, Huang DP (2002) Promoter hypermethylation of the EDNRB gene in nasopharyngeal carcinoma. *Int J Cancer* 98, 651-655.
- [9] Bartsch DK, Fendrich V, Slater EP, Sina-Frey M, Rieder H, Greenhalf W, Chaloupka B, Hahn SA, Neoptolemos JP, Kress R (2005) RNASEL germline variants are associated with pancreatic cancer. *Int J Cancer* 117, 718-722.
- [10] Carpten J, Nupponen N, Isaacs S, Sood R, Robbins C, Xu J, Faruque M, Moses T, Ewing C, Gillanders E, Hu P, Bujnovszky P, Makalowska I, Baffoe-Bonnie A, Faith D, Smith J, Stephan D, Wiley K, Brownstein M, Gildea D, Kelly B, Jenkins R, Hostetter G, Matikainen M, Schleutker J, Klinger K, Connors T, Xiang Y, Wang Z, De Marzo A, Papadopoulos N, Kallionemi OP, Burk R, Meyers D, Gronberg H, Meltzer P, Silverman R, Bailey-Wilson J, Walsh P, Isaacs W, Trent J (2002) Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nat Genet **30**, 181-184.
- [11] Montenegro MF, Ruiz-Espejo F, Campoy FJ, Munoz-Delgado E, de la Cadena MP, Rodriguez-Berrocal FJ, Vidal CJ (2006) Cholinesterases are down-expressed in human colorectal carcinoma. *Cell Mol Life Sci* 63, 2175-2182.
- [12] Mutoh M, Watanabe K, Kitamura T, Shoji Y, Takahashi M, Kawamori T, Tani K, Kobayashi M, Maruyama T, Kobayashi

K, Ohuchida S, Sugimoto Y, Narumiya S, Sugimura T, Wakabayashi K (2002) Involvement of prostaglandin E receptor subtype EP(4) in colon carcinogenesis. *Cancer Res* 62, 28-32.
[13] Alimirah F, Chen J, Davis FJ, Choubey D (2007) IFI16 in

- human prostate cancer. *Mol Cancer Res* 5, 251-259.
 [14] Foran E, McWilliam P, Kelleher D, Croke DT, Long A (2006) The leukocyte protein L-plastin induces proliferation, invasion and loss of E-cadherin expression in colon cancer cells. *Int J Cancer* 118, 2098-2104.
- [15] Liu W, Asa SL, Ezzat S (2005) 1alpha,25-Dihydroxyvitamin D3 targets PTEN-dependent fibronectin expression to restore thyroid cancer cell adhesiveness. *Mol Endocrinol* 19, 2349-2357.
- [16] Sisci D, Aquila S, Middea E, Gentile M, Maggiolini M, Mastroianni F, Montanaro D, Ando S (2004) Fibronectin and type IV collagen activate ERalpha AF-1 by c-Src pathway: Effect on breast cancer cell motility. *Oncogene* 23, 8920-8930.
- [17] Kuivaniemi H, Tromp G, Prockop DJ (1997) Mutations in fibrillar collagens (types I, II, III, and XI), fibril-associated collagen (type IX), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. *Hum Mutat* 9, 300-315.