

**Manganese-induced neurotoxicity in cerebellar granule neurons due to  
perturbation of cell network pathways with potential implications for neurodegenerative disorders**

Raúl Bonne Hernández<sup>1,6,7\*</sup>@, Montserrat Carrascal<sup>2\*</sup>, Joaquin Abian<sup>2</sup>, Bernhard Michalke<sup>3</sup>,  
Marcelo Farina<sup>4</sup>, Yasmilde Rodriguez Gonzalez<sup>5</sup>, Grace O. Iyirhiaro<sup>5</sup>,  
Houman Moteshareie<sup>6</sup> Daniel Burnside<sup>6</sup> Ashkan Golshani<sup>6</sup>, Cristina Suñol<sup>7@</sup>

<sup>1</sup>Laboratory of Bioinorganic and Environmental Toxicology – LABITA. Department of Exact and Earth Sciences. Federal University of São Paulo. Rua Prof. Artur Riedel, 275, CEP: 09972-270. Diadema-SP, Brazil.

<sup>2</sup>CSIC/UAB Proteomics Laboratory, Institut d'Investigacions Biomèdiques de Barcelona, IIBB-CSIC, IDIBAPS, Barcelona, Spain.

<sup>3</sup>Helmholtz Zentrum München GmbH, German Research Center for Environmental Health, Research Unit Analytical BioGeoChemistry, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany.

<sup>4</sup>Departamento de Bioquímica, Centro de Ciências Biológicas, Campus Universitário, Trindade, Bloco C, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, CEP 88040-900, Brazil.

<sup>5</sup>Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa 451 Smyth Road, Ottawa, ON, K1H 8M5

<sup>6</sup>Department of Biology, Carleton University, 209 Nesbitt Biology Building, 1125 Colonel by Drive, Ottawa, ON, K1S 5B6

<sup>7</sup>Department of Neurochemistry and Neuropharmacology, Institut d'Investigacions Biomèdiques de Barcelona, IIBB-CSIC, IDIBAPS, CIBERESP, Barcelona, Spain.

**Table 1:** Proteins identified with differential expression ( $p \leq 0.05$ ), in CGNs under  $MnCl_2$  treatment.

| Protein ID | Protein name                                                                                                                                                                            | Gene name                                             | Length (aa) | MnCl <sub>2</sub> 1 uM |          | MnCl <sub>2</sub> 3 uM |          |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------|-------------|------------------------|----------|------------------------|----------|
|            |                                                                                                                                                                                         |                                                       |             | Ratio                  | P.value  | Ratio                  | Pvalue   |
| A0A087WPL5 | ATP-dependent RNA helicase A                                                                                                                                                            | Dhx9                                                  | 1383        |                        |          | 1.25                   | 0.024121 |
| A1L0U3     | Histone H3 (Fragment)                                                                                                                                                                   | Hist1h3e                                              | 133         | 1.14                   | 0.013644 | 1.43                   | 0.000000 |
| A2ALV1     | Endophilin-A1                                                                                                                                                                           | Sh3gl2                                                | 182         | 0.71                   | 0.028827 |                        |          |
| B2RTP7     | Krt2 protein                                                                                                                                                                            | Krt2                                                  | 707         | 1.39                   | 0.009277 |                        |          |
| B7ZNF6     | Ctnnd2 protein                                                                                                                                                                          | Ctnnd2                                                | 1221        | 1.23                   | 0.032509 | 1.31                   | 0.007870 |
| D3YV7      | Aldose reductase (Fragment)                                                                                                                                                             | Akr1b3                                                | 176         | 1.46                   | 0.001458 |                        |          |
| D3YD05     | Vacuolar protein sorting-associated protein 29 (Vesicle protein sorting 29) (Fragment)                                                                                                  | Vps29                                                 | 124         | 0.89                   | 0.049709 | 0.77                   | 0.002133 |
| E0CZ27     | Histone H3 (Fragment)                                                                                                                                                                   | H3f3a                                                 | 119         | 1.15                   | 0.000260 | 1.22                   | 0.000014 |
| E9PYF1     | Aminopeptidase B                                                                                                                                                                        | Rnpep                                                 | 611         | 0.73                   | 0.006078 |                        |          |
| E9Q7H5     | Uncharacterized protein                                                                                                                                                                 | Gm8991                                                | 300         |                        |          | 1.33                   | 0.002612 |
| E9Q801     | Neural cell adhesion molecule 1                                                                                                                                                         | Ncam1                                                 | 849         |                        |          | 1.21                   | 0.003292 |
| F8WIX8     | Histone H2A                                                                                                                                                                             | Hist1h2a1                                             | 125         |                        |          | 1.22                   | 0.009333 |
| J3QPS8     | Eukaryotic translation initiation factor 5A-1                                                                                                                                           | Eif5a                                                 | 76          | 0.51                   | 0.000160 | 1.41                   | 0.002349 |
| Q08553     | Dihydropyrimidine-related protein 2 (DRP-2) (Unc-33-like phosphoprotein 2) (ULIP-2)                                                                                                     | Dpys12 Crmp2 Ulip2                                    | 572         | 1.28                   | 0.012225 |                        |          |
| Q08585     | Clathrin light chain A (Lca)                                                                                                                                                            | CltA                                                  | 235         |                        |          | 0.92                   | 0.013849 |
| Q08749     | Dihydropyridyl dehydrogenase, mitochondrial (EC 1.8.1.4) (Dihydropyrimidine dehydrogenase)                                                                                              | Dld                                                   | 509         | 1.19                   | 0.040035 | 1.26                   | 0.007697 |
| Q35658     | Complement component 1 Q subcomponent-binding protein, mitochondrial (GC1q-R protein) (Glycoprotein gC1qBP) (C1qBP)                                                                     | C1qbpb Gc1qbpb                                        | 278         | 1.20                   | 0.045773 | 1.31                   | 0.004375 |
| P02301     | Histone H3.3C (Embryonic)                                                                                                                                                               | H3f3c Gm14384                                         | 136         | 1.38                   | 0.001590 |                        |          |
| P05064     | Fructose-bisphosphate aldolase A (EC 4.1.1.13) (Aldolase 1) (Muscle-type aldolase)                                                                                                      | Aldoa Aldo1                                           | 364         |                        |          | 1.22                   | 0.012392 |
| P07724     | Serum albumin                                                                                                                                                                           | Alb Alb-1 Alb1                                        | 608         | 0.78                   | 0.000652 | 0.55                   | 0.000000 |
| P08249     | Malate dehydrogenase, mitochondrial (EC 1.1.1.37)                                                                                                                                       | Mdh2 Mor1                                             | 338         | 1.22                   | 0.002639 | 1.30                   | 0.000232 |
| P12787     | Cytochrome c oxidase subunit 5A, mitochondrial (Cytochrome c oxidase polypeptide Va)                                                                                                    | Cox5a                                                 | 146         |                        |          | 1.21                   | 0.021326 |
| P20065-2   | Thymosin beta-4 (T beta 4) [Cleaved into: Hematopoietic system regulatory peptide (Seraspenide)]                                                                                        | Tmsb4x Ptmb4 Tmsb4                                    | 50          |                        |          | 0.74                   | 0.011237 |
| P28184     | Metallothionein-3 (MT-3) (Growth inhibitory factor) (GIF) (Metallothionein-III) (MT-III)                                                                                                | Mt3                                                   | 68          |                        |          | 0.72                   | 0.011238 |
| P31786     | Acyl-CoA-binding protein (ACBP) (Diazepam-binding inhibitor) (DBI) (Endozepine) (EP)                                                                                                    | Dbi                                                   | 87          |                        |          | 0.71                   | 0.002068 |
| P48428     | Tubulin-specific chaperone A (TCP1-chaperonin cofactor A) (Tubulin-folding cofactor A) (CFA)                                                                                            | Tbca                                                  | 108         | 0.83                   | 0.006337 | 0.69                   | 0.000016 |
| P48678-3   | Prelamin-A/C [Cleaved into: Lamin-A/C]                                                                                                                                                  | Lmna Lmn1                                             | 665         |                        |          | 1.27                   | 0.014651 |
| P50114     | Protein S100-B (S-100 protein subunit beta) (S100 calcium-binding protein B)                                                                                                            | S100b                                                 | 92          |                        |          | 0.76                   | 0.026235 |
| P54227     | Stathmin (Leukemia-associated gene protein) (Leukemia-associated phosphoprotein p18) (Metablastin) (Oncoprotein 18) (Op18) (Phosphoprotein p19) (pp19) (Prosolin) (Protein Pr22) (pp17) | Stmn1 Lag Lap18 Pr22                                  | 149         | 1.50                   | 0.000000 | 1.24                   | 0.000477 |
| P62204     | Calmodulin (CaM)                                                                                                                                                                        | Calm1 Calm Cam Cam1; Calm2 Cam2 Camb; Calm3 Cam3 Camc | 149         | 0.78                   | 0.028164 |                        |          |
| P68373     | Tubulin alpha-1C chain (Alpha-tubulin 6) (Alpha-tubulin isotype M-alpha-6) (Tubulin alpha-6 chain)                                                                                      | Tuba1c Tuba6                                          | 449         |                        |          | 1.22                   | 0.026236 |
| Q04447     | Creatine kinase B-type (EC 2.7.3.2) (B-CK) (Creatine kinase B chain)                                                                                                                    | Ckb Kcbb                                              | 381         |                        |          | 0.76                   | 0.032190 |
| Q06185     | ATP synthase subunit e, mitochondrial (ATPase subunit e)                                                                                                                                | Atp5i Atp5k Lfm-1 Lfm1                                | 71          | 1.15                   | 0.000636 | 1.29                   | 0.000035 |
| Q0VDP2     | Slc2a13 protein                                                                                                                                                                         | Slc2a13                                               | 504         |                        |          | 1.82                   | 0.036362 |
| Q148V7-3   | LisH domain and HEAT repeat-containing protein KIAA1468                                                                                                                                 | Kiaa1468                                              | 1216        |                        |          | 1.24                   | 0.034789 |
| Q3TD08     | Putative uncharacterized protein                                                                                                                                                        | Eif4b                                                 | 609         |                        |          | 1.24                   | 0.007756 |
| Q3TSX8     | Putative uncharacterized protein                                                                                                                                                        | Tomm70a                                               | 611         | 1.26                   | 0.010328 |                        |          |
| Q3U0V1     | Far upstream element-binding protein 2 (FUSE-binding protein 2) (KH type-splicing regulatory protein) (KSRP)                                                                            | Khsrp Fubp2                                           | 748         | 1.20                   | 0.000268 | 1.17                   | 0.001354 |
| Q3UDY7     | Putative uncharacterized protein (Fragment)                                                                                                                                             | Dpp3                                                  | 583         |                        |          | 3.93                   | 0.023789 |
| Q3UGT5     | Alpha-1,4 glucan phosphorylase (EC 2.4.1.1)                                                                                                                                             | Pygb                                                  | 843         | 1.20                   | 0.025901 |                        |          |
| Q3UXS6     | Putative uncharacterized protein                                                                                                                                                        | Rps15                                                 | 124         |                        |          | 1.51                   | 0.038979 |
| Q3V1L4     | Cytosolic purine 5'-nucleotidase (EC 3.1.3.5) (Cytosolic 5'-nucleotidase II)                                                                                                            | Nt5c2                                                 | 560         |                        |          | 0.77                   | 0.035912 |
| Q56920     | Ribosomal protein L14                                                                                                                                                                   | Rpl14-ps1 Rpl14                                       | 216         | 1.35                   | 0.015797 |                        |          |
| Q5D0A4     | Stx1a protein (Fragment)                                                                                                                                                                | Stx1a                                                 | 287         | 1.10                   | 0.040216 | 1.22                   | 0.000114 |
| Q61699     | Heat shock protein 105 kDa (42 degrees C-HSP) (Heat shock 110 kDa protein) (Heat shock-related 100 kDa protein E71) (HSP-E71)                                                           | Hspb1 Hsp105 Hsp110 Kiaa0201                          | 858         | 0.87                   | 0.012619 | 0.78                   | 0.000150 |
| Q62261     | Spectrin beta chain, non-erythrocytic 1 (Beta-II spectrin) (Embryonic liver fodrin) (Fodrin beta chain)                                                                                 | Sptbn1 Elf Spnb-2 Spnb2 Sptb2                         | 2363        | 1.21                   | 0.002752 | 1.20                   | 0.003695 |
| Q62425     | Cytochrome c oxidase subunit NDUF4A                                                                                                                                                     | Ndufa4                                                | 82          | 1.15                   | 0.000375 | 1.21                   | 0.000017 |
| Q68FD6     | Tln2 protein                                                                                                                                                                            | Tln2                                                  | 1775        |                        |          | 1.22                   | 0.001702 |
| Q80U51     | MKIAA0275 protein (Fragment)                                                                                                                                                            | Spock2 mKIAA0275                                      | 380         |                        |          | 1.25                   | 0.001090 |
| Q88GB7-2   | Enolase-phosphatase E1 (EC 3.1.3.77) (2,3-diketo-5-methylthio-1-phosphopentane phosphatase) (MASA homolog)                                                                              | Enoph1 Masa                                           | 257         | 0.83                   | 0.001195 | 0.78                   | 0.000286 |
| Q8BM51     | Trifunctional enzyme subunit alpha, mitochondrial (TP-alpha) [Includes: Long-chain enoyl-CoA hydratase (EC 4.2.1.17); Long chain 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.211)]        | Hadha                                                 | 763         | 0.78                   | 0.002571 | 0.88                   | 0.032362 |
| Q8C129     | Putative uncharacterized protein                                                                                                                                                        |                                                       | 159         | 1.20                   | 0.037250 | 1.20                   | 0.037243 |
| Q8C553     | Putative uncharacterized protein (Fragment)                                                                                                                                             | Lmnb1                                                 | 413         |                        |          | 1.21                   | 0.000712 |
| Q8CE19     | Putative uncharacterized protein                                                                                                                                                        | Syn2                                                  | 313         | 1.41                   | 0.000072 | 1.41                   | 0.000072 |
| Q91X76     | 5'-nucleotidase domain containing 2 (Protein Nt5dc2)                                                                                                                                    | Nt5dc2                                                | 390         |                        |          | 1.24                   | 0.018585 |
| Q92129     | Mannose-6-phosphate isomerase (EC 5.3.1.8)                                                                                                                                              | Mpi                                                   | 423         | 0.76                   | 0.032892 | 0.78                   | 0.045094 |
| Q922R8     | Protein disulfide-isomerase A6 (EC 5.3.4.1) (Thioredoxin domain-containing protein 7)                                                                                                   | Pdia6 Txndc7                                          | 440         | 0.65                   | 0.041838 |                        |          |
| Q9R1P1     | Proteasome subunit beta type-3 (EC 3.4.25.1) (Proteasome chain 13) (Proteasome component C10-II) (Proteasome theta chain)                                                               | Psmb3                                                 | 205         |                        |          | 0.72                   | 0.000952 |
| Q9VV02-2   | RNA-binding motif protein, X chromosome (Heterogeneous nuclear ribonucleoprotein G) (hnRNP G) [Cleaved into: RNA-binding motif protein, X chromosome, N-terminally processed]           | RbmX Hnrrpg Hnrrp RbmXp1 RbmXrt                       | 391         | 1.17                   | 0.003830 | 1.26                   | 0.000126 |

Pink: Up-expression, Green: Repression.

**Table 2:** Proteins identified with differential expression ( $p \leq 0.05$ ), in CGNs under Maneb treatment.

| Protein ID | Protein name                                                                                                                                                                                                                                                                         | Gene name             | Length (aa) | MB1 1 uM |             | MB3   |             |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|-------------|----------|-------------|-------|-------------|
|            |                                                                                                                                                                                                                                                                                      |                       |             | Ratio    | P.value     | Ratio | Pvalue      |
| A110U3     | Histone H3 (Fragment)                                                                                                                                                                                                                                                                | Hist1h3e              | 133         | 1.15     | 0.008060958 | 1.22  | 0.000476574 |
| A2AE89     | Glutathione S-transferase Mu 1 (Fragment)                                                                                                                                                                                                                                            | Gstm1                 | 201         | 1.14     | 0.018570537 | 1.30  | 3.96619E-05 |
| A2AKT6     | Exportin-1 (Fragment)                                                                                                                                                                                                                                                                | Xpo1                  | 59          | 1.16     | 0.046640882 | 1.27  | 0.009460814 |
| B1ARAS     | 60S ribosomal protein L26                                                                                                                                                                                                                                                            | Rpl26                 | 143         | 1.23     | 0.022545763 |       |             |
| B1AXY1     | DnaJ homolog subfamily A member 1 (Fragment)                                                                                                                                                                                                                                         | DnaJ1                 | 278         | 1.14     | 0.010189631 |       |             |
| D3YU12     | NmrA-like family domain-containing protein 1                                                                                                                                                                                                                                         | NmrA1                 | 299         | 0.73     | 0.022167987 |       |             |
| D3Z228     | Neurocalcin-delta (Fragment)                                                                                                                                                                                                                                                         | Ncald                 | 163         | 1.20     | 0.010435521 | 1.22  | 0.005323493 |
| D3Z4S6     | Transmembrane protein 132A                                                                                                                                                                                                                                                           | Tmem132a              | 687         | 0.78     | 0.037087955 |       |             |
| E0CKA0     | Hepatoma-derived growth factor (Fragment)                                                                                                                                                                                                                                            | Hdgf                  | 202         |          |             | 0.67  | 0.001768809 |
| E9Q7G1     | Protein Tmed7                                                                                                                                                                                                                                                                        | Tmed7                 | 188         |          |             | 1.21  | 0.024820389 |
| G3X9K3     | Brefeldin A-inhibited guanine nucleotide-exchange protein 1 (BIG1) [Brefeldin A-inhibited GEP 1] [ADP-ribosylation factor guanine nucleotide-exchange factor 1]                                                                                                                      | Arfgef1               | 1846        |          |             | 0.79  | 0.040183299 |
| O35737     | Heterogeneous nuclear ribonucleoprotein H (hnRNP H) [Cleaved into: Heterogeneous nuclear ribonucleoprotein H, N-terminally processed]                                                                                                                                                | Hnrnp1 Hnrph Hnrph1   | 449         |          |             | 1.20  | 0.038901811 |
| O35864     | COP9 signalosome complex subunit 5 (SGN5) [Signalosome subunit 5] (Jun activation domain-binding protein 1) [Kip1 C-terminus-interacting protein 2]                                                                                                                                  | Cops5 Csn5 Jab1 Kic2  | 334         |          |             | 1.32  | 0.011158335 |
| P08752     | Guanine nucleotide-binding protein (Gβ) subunit alpha-2 (Adenylate cyclase-inhibiting G alpha protein)                                                                                                                                                                               | Gnai2 Gnai-2          | 355         | 1.31     | 0.048569527 |       |             |
| P09671     | Superoxide dismutase [Mn], mitochondrial (EC 1.15.1.1)                                                                                                                                                                                                                               | Sod2 Sod-2            | 222         | 1.29     | 0.000221229 | 1.44  | 2.75645E-06 |
| P12787     | Cytochrome c oxidase subunit 5A, mitochondrial (Cytochrome c oxidase polypeptide Va)                                                                                                                                                                                                 | Cox5a                 | 146         | 1.20     | 0.02115515  |       |             |
| P16014     | Secretogranin-1 (Chromogranin-8) [Cg8] [Secretogranin I] [Sg1] [Cleaved into: CCB peptide; PE-11]                                                                                                                                                                                    | Chgb Scg-1 Scg1       | 677         |          |             | 0.74  | 0.001718356 |
| P18760     | Cofilin-1 (Cofilin, non-muscle isoform)                                                                                                                                                                                                                                              | Cfl1                  | 166         | 1.26     | 0.002641267 |       |             |
| P20152     | Vimentin                                                                                                                                                                                                                                                                             | Vim                   | 466         | 1.22     | 0.004194657 |       |             |
| P27773     | Protein disulfide-isomerase A3 (EC 5.3.4.1) [S8 kDa glucose-regulated protein] [S8 kDa microsomal protein] [p58] [Disulfide isomerase ER-60] [Endoplasmic reticulum resident protein 57] [ER protein 57] [ERp57] [Endoplasmic reticulum resident protein 60] [ER protein 60] [ERp60] | Pdia3 Erp Erp60 Grp58 | 505         |          |             | 0.73  | 0.042898436 |
| P29595     | NEDD8 (Neddylin) [Neural precursor cell expressed developmentally down-regulated protein 8] [NEDD-8] [Ubiquitin-like protein Nedd8]                                                                                                                                                  | Nedd8 Nedd-8          | 81          | 0.84     | 0.002207062 | 0.91  | 0.041190864 |
| P31786     | Acyl-CoA-binding protein (ACBP) [Diazepam-binding inhibitor] [DBI] [Endozepine] (EP)                                                                                                                                                                                                 | Dbi                   | 87          | 1.53     | 0.000139255 |       |             |
| P32261     | Antithrombin-III (ATIII) [Serpin C1]                                                                                                                                                                                                                                                 | Serpinc1 At3          | 465         | 1.09     | 0.017990049 | 1.41  | 2.27854E-08 |
| P48678-3   | Prelamin-A/C [Cleaved into: Lamin-A/C]                                                                                                                                                                                                                                               | Lmna Lmn1             | 665         | 1.24     | 0.023476736 |       |             |
| Q05CD8     | Reticulon                                                                                                                                                                                                                                                                            | Rtn1                  | 68          |          |             | 1.36  | 0.01375154  |
| Q3TL33     | Putative uncharacterized protein                                                                                                                                                                                                                                                     | Calu                  | 315         | 1.32     | 0.047790699 |       |             |
| Q3TPC5     | Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B (Fragment)                                                                                                                                                                                                       | Ppp2r2a               | 403         | 1.31     | 0.02595407  |       |             |
| Q3UDY7     | Putative uncharacterized protein (Fragment)                                                                                                                                                                                                                                          | Dpp3                  | 583         | 0.23     | 0.013550008 |       |             |
| Q3UUG6-2   | TBC1 domain family member 24                                                                                                                                                                                                                                                         | Tbc1d24 Kiaa1171      | 561         |          |             | 1.22  | 0.025641912 |
| Q58EV2     | Apoa1 protein                                                                                                                                                                                                                                                                        | Apoa1                 | 198         |          |             | 1.44  | 0.017235949 |
| Q61792     | LIM and SH3 domain protein 1 (LASP-1) [Metastatic lymph node gene 50 protein] (MLN 50)                                                                                                                                                                                               | Lasp1 Mln50           | 263         |          |             | 1.21  | 0.014251716 |
| Q64669     | NAD(P)H dehydrogenase [quinone] 1 (EC 1.6.5.2) [Azooreductase] [DT-diaphorase] [DTD] [Menadiene reductase] [NAD(P)H:quinone oxidoreductase 1] [Phylloquinone reductase] [Quinone reductase 1] (QR1)                                                                                  | Nqo1 Dia4 Nmo1 Nmor1  | 274         | 1.22     | 0.031992737 | 1.42  | 0.003333368 |
| Q6DFW4     | Nucleolar protein 58 (MSSP) [Nucleolar protein 5] [SIK-similar protein]                                                                                                                                                                                                              | Nop58 Nol5            | 536         | 0.65     | 0.012680475 |       |             |
| Q6GQT1     | Alpha-2-macroglobulin-P (Alpha-2-macroglobulin)                                                                                                                                                                                                                                      | A2mp A2m              | 1474        |          |             | 1.55  | 2.07343E-05 |
| Q6KAM8     | MFLJ00343 protein (Fragment)                                                                                                                                                                                                                                                         | Flna mFLJ00343        | 1952        | 0.69     | 0.01555104  |       |             |
| Q7TM10     | Psm11 protein (Fragment)                                                                                                                                                                                                                                                             | Psm11                 | 416         | 4.33     | 0.043283597 |       |             |
| Q7TM8-2    | E3 ubiquitin-protein ligase HUWE1 (EC 6.3.2.-) (E3Histone) (HECT, UBA and WWE domain-containing protein 1) [Upstream regulatory element-binding protein 1] (URE-B1) [URE-binding protein 1]                                                                                          | Huwe1 Kiaa0312 Ureb1  | 4377        |          |             | 0.79  | 0.004585944 |
| Q80YA7     | Dipeptidyl peptidase 8 (DPP8) (EC 3.4.14.5) [Dipeptidyl peptidase VIII] [DPP VIII]                                                                                                                                                                                                   | Dpp8                  | 892         | 1.64     | 0.043769497 |       |             |
| Q8BMX6     | Putative uncharacterized protein (Fragment)                                                                                                                                                                                                                                          | Ppid                  | 109         | 1.21     | 0.014178739 | 1.25  | 0.005849781 |
| Q8BU30     | Isoleucine-tRNA ligase, cytoplasmic (EC 6.1.1.5) [Isoleucyl-tRNA synthetase] (IRS) (IleRS)                                                                                                                                                                                           | Iars                  | 1262        |          |             | 0.75  | 0.012572707 |
| Q8CIF2     | 10-formyltetrahydrofolate dehydrogenase (EC 1.5.1.6)                                                                                                                                                                                                                                 | Aldh1l1 Fthd1         | 902         |          |             | 0.74  | 0.03674741  |
| Q8K0C9     | GDP-mannose 4,6 dehydratase (EC 4.2.1.47) (GDP-D-mannose dehydratase) (GMD)                                                                                                                                                                                                          | Gmds                  | 372         | 0.77     | 0.039929439 |       |             |
| Q91V61-2   | Sideroflexin-3                                                                                                                                                                                                                                                                       | Sfk3                  | 321         | 0.77     | 7.54367E-05 | 0.87  | 0.006873719 |
| Q91VM5     | RNA binding motif protein, X-linked-like-1 (Heterogeneous nuclear ribonucleoprotein G-like 1) [RNA binding motif protein, X chromosome retrogene]                                                                                                                                    | Rbm11 Rbmxt           | 388         |          |             | 1.34  | 0.031646938 |
| Q91X76     | 5'-nucleotidase domain containing 2 (Protein NT5dc2)                                                                                                                                                                                                                                 | Nt5dc2                | 390         | 0.78     | 0.008660765 |       |             |
| Q91Y12     | Sorting nexin-4                                                                                                                                                                                                                                                                      | Snx4                  | 450         |          |             | 1.31  | 0.047718403 |
| Q99PL5-2   | Ribosome-binding protein 1 (Ribosome receptor protein) (RRp) (mRRp)                                                                                                                                                                                                                  | Rrbp1                 | 1605        |          |             | 0.75  | 0.014820026 |
| Q9D172     | ES1 protein homolog, mitochondrial                                                                                                                                                                                                                                                   | D10Ihu81e             | 266         | 1.21     | 0.001555807 |       |             |
| Q9D586     | Ciliary neurotrophic factor receptor subunit alpha                                                                                                                                                                                                                                   | Cntrf                 | 113         | 1.27     | 0.011343075 |       |             |
| Q9D7N9     | Adipocyte plasma membrane-associated protein (Protein DD16)                                                                                                                                                                                                                          | Apmap                 | 415         |          |             | 0.72  | 0.018158364 |
| Q9DC69     | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial (Complex I-39kD) (CI-39kD) [NADH-ubiquinone oxidoreductase 39 kDa subunit]                                                                                                                               | Ndufa9                | 377         |          |             | 0.79  | 0.023740626 |

Pink: Up-expression, Green: Repression

**Table 3** The Pathways affected by MnCl<sub>2</sub> in CGNs

| CELL PROCESSES             | PATHWAYS                                                                                                 | OBSERVED GENES | FDR      |
|----------------------------|----------------------------------------------------------------------------------------------------------|----------------|----------|
| METABOLISM OF PROTEINS     | Ribosome                                                                                                 | 51             | 4.03E-67 |
|                            | Metabolism of RNA                                                                                        | 37             | 5.53E-25 |
|                            | Ribosomal scanning and start codon recognition                                                           | 19             | 9.76E-24 |
|                            | Proteasome                                                                                               | 19             | 1.96E-24 |
|                            | Translation initiation complex formation                                                                 | 19             | 1.26E-23 |
|                            | L13a-mediated translational silencing of Ceruloplasmin expression                                        | 19             | 1.43E-23 |
|                            | Formation of a pool of free 40S subunits                                                                 | 17             | 2.45E-22 |
|                            | AUF1 (hnRNP D0) binds and destabilizes mRNA                                                              | 18             | 6.24E-22 |
|                            | Formation of the ternary complex and subsequently the 43S complex                                        | 17             | 6.24E-22 |
|                            | Regulation of mRNA stability by proteins that bind AU-rich elements                                      | 19             | 3.63E-21 |
|                            | Metabolism of polyamines                                                                                 | 19             | 4.90E-21 |
|                            | Autodegradation of the E3 ubiquitin ligase COP1                                                          | 17             | 3.63E-21 |
|                            | Ubiquitin-dependent degradation of Cyclin D1                                                             | 17             | 3.63E-21 |
|                            | Ubiquitin Mediated Degradation of Phosphorylated Cdc25A                                                  | 17             | 3.63E-21 |
|                            | Regulation of ornithine decarboxylase (ODC)                                                              | 17             | 3.64E-21 |
|                            | Degradation of AXIN                                                                                      | 17             | 4.64E-21 |
|                            | SCF-beta-TrCP mediated degradation of Emi1                                                               | 17             | 5.41E-21 |
|                            | Degradation of DVL                                                                                       | 17             | 8.40E-21 |
|                            | Degradation of GLI1 by the proteasome                                                                    | 17             | 8.40E-21 |
|                            | Downstream signaling events of B Cell Receptor (BCR)                                                     | 18             | 9.20E-21 |
|                            | Metabolism of amino acids and derivatives                                                                | 24             | 1.81E-19 |
|                            | Metabolism of proteins                                                                                   | 44             | 2.65E-15 |
|                            | Post-translational protein modification                                                                  | 25             | 6.34E-06 |
| CELL METABOLISM            | Oxidative phosphorylation                                                                                | 18             | 7.77E-16 |
|                            | Metabolism                                                                                               | 40             | 4.94E-11 |
|                            | Metabolic pathways                                                                                       | 33             | 3.37E-09 |
|                            | The citric acid (TCA) cycle and respiratory electron transport                                           | 12             | 1.30E-09 |
|                            | Formation of ATP by chemiosmotic coupling                                                                | 6              | 3.43E-08 |
|                            | Pyruvate metabolism and Citric Acid (TCA) cycle                                                          | 6              | 9.05E-06 |
|                            | Glyoxylate metabolism and glycine degradation                                                            | 5              | 9.80E-06 |
|                            | Carbon metabolism                                                                                        | 8              | 3.58E-05 |
|                            | Citrate cycle (TCA cycle)                                                                                | 5              | 6.00E-05 |
|                            | Regulation of pyruvate dehydrogenase (PDH) complex                                                       | 4              | 3.08E-05 |
| NEUROTRANSMITTER SYSTEM    | Serotonin Neurotransmitter Release Cycle                                                                 | 6              | 2.57E-08 |
|                            | Dopamine Neurotransmitter Release Cycle                                                                  | 6              | 9.68E-08 |
|                            | Acetylcholine Neurotransmitter Release Cycle                                                             | 5              | 4.14E-07 |
|                            | Norepinephrine Neurotransmitter Release Cycle                                                            | 5              | 9.60E-07 |
|                            | GABA synthesis release reuptake and degradation                                                          | 5              | 9.60E-07 |
|                            | Glutamate Neurotransmitter Release Cycle                                                                 | 5              | 2.93E-06 |
|                            | Transmission across Chemical Synapses                                                                    | 7              | 1.30E-03 |
|                            | Synaptic vesicle cycle                                                                                   | 5              | 1.00E-03 |
| CELL SIGNALING             | Downstream signaling events of B Cell Receptor (BCR)                                                     | 18             | 9.20E-21 |
|                            | Dectin-1 mediated noncanonical NF-kB signaling                                                           | 17             | 9.93E-21 |
|                            | NIK-->noncanonical NF-kB signaling                                                                       | 17             | 9.93E-21 |
|                            | CLEC7A (Dectin-1) signaling                                                                              | 18             | 1.56E-19 |
|                            | MAPK6/MAPK4 signaling                                                                                    | 17             | 1.56E-19 |
|                            | Interleukin-1 signaling                                                                                  | 18             | 2.11E-19 |
|                            | Downstream TCR signaling                                                                                 | 17             | 1.95E-18 |
|                            | Fc epsilon receptor (FCER1) signaling                                                                    | 18             | 5.08E-18 |
|                            | Signaling by WNT                                                                                         | 23             | 6.38E-18 |
|                            | Beta-catenin independent WNT signaling                                                                   | 18             | 1.76E-17 |
|                            | TCF dependent signaling in response to WNT                                                               | 19             | 3.00E-16 |
|                            | RAF/MAP kinase cascade                                                                                   | 19             | 1.80E-14 |
|                            | Signaling by Interleukins                                                                                | 21             | 4.64E-15 |
|                            | Cytokine Signaling in Immune system                                                                      | 22             | 1.37E-13 |
|                            | Intracellular signaling by second messengers                                                             | 18             | 5.96E-13 |
|                            | Signal Transduction                                                                                      | 32             | 1.40E-03 |
|                            | Signaling by Nuclear Receptors                                                                           | 6              | 1.40E-03 |
|                            | Signaling by Retinoic Acid                                                                               | 4              | 2.20E-03 |
| IMMUNE SYSTEM              | Adaptive Immune System                                                                                   | 20             | 2.15E-07 |
|                            | Innate Immune System                                                                                     | 22             | 1.33E-06 |
|                            | Immune System                                                                                            | 27             | 3.65E-05 |
| CELL CYCLE                 | Ubiquitin Mediated Degradation of Phosphorylated Cdc25A                                                  | 17             | 3.63E-21 |
|                            | CDT1 association with the CDC6:ORC:origin complex                                                        | 17             | 9.93E-21 |
|                            | APC/C:Cdc20 mediated degradation of Securin                                                              | 17             | 5.13E-20 |
|                            | APC/C:Cdh1 mediated degradation of Cdc20 and other APC/C:Cdh1 targeted proteins in late mitosis/early G1 | 17             | 1.32E-19 |
|                            | Cdc20:Phospho-APC/C mediated degradation of Cyclin A                                                     | 17             | 1.32E-19 |
|                            | CDK-mediated phosphorylation and removal of Cdc6                                                         | 17             | 1.32E-19 |
|                            | CDK-mediated phosphorylation and removal of Cdc6                                                         | 17             | 1.32E-19 |
|                            | G2/M Checkpoints                                                                                         | 17             | 1.41E-15 |
|                            | M Phase                                                                                                  | 21             | 1.42E-13 |
| NEURODEGENERATIVE DISEASES | Alzheimer's disease                                                                                      | 18             | 3.66E-14 |
|                            | Parkinson's disease                                                                                      | 16             | 3.54E-13 |
|                            | Huntington's disease                                                                                     | 16             | 2.29E-11 |

**Table 4:** The Pathways affected by Maneb in CGNs

| CELL PROCESSES             | PATHWAYS                                                                                                 | OBSERVED GENES | FDR      |
|----------------------------|----------------------------------------------------------------------------------------------------------|----------------|----------|
| METABOLISM OF PROTEINS     | Ribosome                                                                                                 | 55             | 8.32E-76 |
|                            | Metabolism of RNA                                                                                        | 38             | 5.20E-27 |
|                            | Formation of a pool of free 40S subunits                                                                 | 18             | 7.69E-24 |
|                            | Formation of the ternary complex and subsequently the 43S complex                                        | 18             | 1.57E-23 |
|                            | Ribosomal scanning and start codon recognition                                                           | 18             | 9.13E-23 |
|                            | Translation initiation complex formation                                                                 | 18             | 1.37E-22 |
|                            | L13a-mediated translational silencing of Ceruloplasmin expression                                        | 18             | 1.80E-22 |
|                            | Major pathway of rRNA processing in the nucleolus and cytosol                                            | 21             | 4.74E-22 |
|                            | Proteasome                                                                                               | 14             | 3.11E-17 |
|                            | Metabolism of proteins                                                                                   | 47             | 1.06E-17 |
|                            | Ubiquitin-dependent degradation of Cyclin D1                                                             | 14             | 7.63E-17 |
|                            | Metabolism of polyamines                                                                                 | 15             | 7.20E-16 |
|                            | Metabolism of amino acids and derivatives                                                                | 16             | 4.06E-11 |
|                            | Post-translational protein modification                                                                  | 29             | 1.34E-08 |
|                            | N-glycan trimming in the ER and Calnexin/Calreticulin cycle                                              | 4              | 2.60E-04 |
|                            | Post-translational protein phosphorylation                                                               | 5              | 3.90E-03 |
|                            | Ribosome biogenesis in eukaryotes                                                                        | 4              | 1.78E-02 |
| CELL METABOLISM            | Oxidative phosphorylation                                                                                | 23             | 3.78E-23 |
|                            | Metabolic pathways                                                                                       | 25             | 2.70E-05 |
|                            | Complex I biogenesis                                                                                     | 17             | 7.49E-21 |
| CELL SIGNALING             | Retrograde endocannabinoid signaling                                                                     | 19             | 3.21E-17 |
|                            | MAPK6/MAPK4 signaling                                                                                    | 15             | 1.40E-16 |
|                            | Dectin-1 mediated noncanonical NF-kB signaling                                                           | 14             | 2.07E-16 |
|                            | NIK-->noncanonical NF-kB signaling                                                                       | 14             | 2.07E-16 |
|                            | Downstream TCR signaling                                                                                 | 14             | 1.58E-14 |
|                            | Interleukin-1 signaling                                                                                  | 14             | 3.09E-14 |
|                            | TCF dependent signaling in response to WNT                                                               | 15             | 6.59E-12 |
| IMMUNE SYSTEM              | Signaling by Interleukins                                                                                | 15             | 2.48E-09 |
|                            | Activation of NF-kappaB in B cells                                                                       | 14             | 4.85E-16 |
|                            | Innate Immune System                                                                                     | 16             | 1.20E-03 |
|                            | Immune System                                                                                            | 19             | 2.28E-02 |
|                            | Antigen processing-Cross presentation                                                                    | 15             | 7.72E-16 |
|                            | Cross-presentation of soluble exogenous antigens (endosomes)                                             | 13             | 8.75E-16 |
|                            | Degradation of beta-catenin by the destruction complex                                                   | 14             | 3.95E-15 |
| CELL CYCLE                 | FCER1 mediated NF-kB activation                                                                          | 14             | 3.95E-15 |
|                            | Ubiquitin Mediated Degradation of Phosphorylated Cdc25A                                                  | 14             | 8.37E-17 |
|                            | Regulation of ornithine decarboxylase (ODC)                                                              | 14             | 8.96E-17 |
|                            | FBXL7 down-regulates AURKA during mitotic entry and in early mitosis                                     | 14             | 1.30E-16 |
|                            | CDT1 association with the CDC6:ORC:origin complex                                                        | 14             | 2.07E-16 |
|                            | APC/C:Cdc20 mediated degradation of Securin                                                              | 14             | 7.69E-16 |
|                            | APC/C:Cdh1 mediated degradation of Cdc20 and other APC/C:Cdh1 targeted proteins in late mitosis/early G1 | 14             | 1.60E-15 |
|                            | Cdc20:Phospho-APC/C mediated degradation of Cyclin A                                                     | 14             | 1.60E-15 |
|                            | CDK-mediated phosphorylation and removal of Cdc6                                                         | 14             | 1.60E-15 |
|                            | The role of GTSE1 in G2/M progression after G2 checkpoint                                                | 14             | 2.10E-15 |
|                            | Mitotic G1-G1/S phases                                                                                   | 15             | 1.05E-13 |
|                            | Separation of Sister Chromatids                                                                          | 16             | 6.52E-13 |
|                            | G2/M Transition                                                                                          | 16             | 6.52E-13 |
|                            | G2/M Checkpoints                                                                                         | 14             | 3.56E-12 |
|                            | Cell Cycle Checkpoints                                                                                   | 16             | 8.22E-11 |
|                            | Cell Cycle Mitotic                                                                                       | 17             | 4.10E-08 |
|                            | Cell Cycle                                                                                               | 18             | 4.40E-08 |
| NEURODEGENERATIVE DISEASES | Cyclin A/B1/B2 associated events during G2/M transition                                                  | 2              | 3.39E-02 |
|                            | Parkinson's disease                                                                                      | 24             | 8.72E-24 |
|                            | Huntington's disease                                                                                     | 24             | 2.33E-21 |
|                            | Alzheimer's disease                                                                                      | 23             | 3.74E-21 |

**Table 5:** List of genes for validation proteomic data

| <i>Symbol</i> | <i>Gene Name</i>                            | Sequence (5'→3') |                             | Template strand | Length | Start     | Stop     | Tm    | GC %  | Self complementarity | Self 3' complementarity |
|---------------|---------------------------------------------|------------------|-----------------------------|-----------------|--------|-----------|----------|-------|-------|----------------------|-------------------------|
| <b>Rps15</b>  | <i>ribosomal protein S15</i>                | <b>F</b>         | AAAGTGAGTTGTGGTCCGGG        | Plus            | 20     | 80293874  | 80293893 | 60.18 | 55    | 4                    | 3                       |
|               |                                             | <b>R</b>         | GAGAACTCGCCAGGTAGTG         | Minus           | 20     | 80294001  | 80293982 | 59.83 | 60    | 4                    | 1                       |
|               |                                             | <b>Length</b>    | 128                         |                 |        |           |          |       |       |                      |                         |
| <b>Rpl14</b>  | <i>ribosomal protein L14</i>                | <b>F</b>         | TACGAAATGGGCAGCCACAA        | Plus            | 20     | 120573624 | 1.21E+08 | 60.25 | 50    | 5                    | 3                       |
|               |                                             | <b>R</b>         | TGCTGGCCCTAACCTATCAAG       | Minus           | 21     | 120573937 | 1.21E+08 | 59.51 | 52.38 | 4                    | 1                       |
|               |                                             | <b>Length</b>    | 314                         |                 |        |           |          |       |       |                      |                         |
| Rpl26         | ribosomal protein L26                       | <b>F</b>         | GGCAAGTGGTCCAAGTGTA         | Plus            | 20     | 68903212  | 68903231 | 59.89 | 55    | 5                    | 2                       |
|               |                                             | <b>R</b>         | ACTTGCAAGTGCCATGTAAAC       | Minus           | 21     | 68903388  | 68903368 | 59.39 | 47.62 | 6                    | 2                       |
|               |                                             | <b>Length</b>    | 177                         |                 |        |           |          |       |       |                      |                         |
| Eif4b         | eukaryotic translation initiation factor 4B | <b>F</b>         | GTGATGCCCTATTCCCCTG         | Plus            | 20     | 102092916 | 1.02E+08 | 59.89 | 60    | 2                    | 1                       |
|               |                                             | <b>R</b>         | CCTCAGGGCACCTCCACTAA        | Minus           | 20     | 102093245 | 1.02E+08 | 60.91 | 60    | 5                    | 2                       |
|               |                                             | <b>Length</b>    | 330                         |                 |        |           |          |       |       |                      |                         |
| Eif5a         | eukaryotic translation initiation factor 5A | <b>F</b>         | CACGGGTCTTAGTACGCAGG        | Plus            | 20     | 25812     | 25831    | 60.18 | 60    | 4                    | 2                       |
|               |                                             | <b>R</b>         | TTGGAAGCACTACGGACTGG        | Minus           | 20     | 26021     | 26002    | 59.68 | 55    | 4                    | 2                       |
|               |                                             | <b>Length</b>    | 210                         |                 |        |           |          |       |       |                      |                         |
| Nop58         | NOP58 ribonucleoprotein                     | <b>F</b>         | TTCCGAGGAGGAGCCATGTA        | Plus            | 20     | 59710616  | 59710635 | 60.03 | 55    | 4                    | 2                       |
|               |                                             | <b>R</b>         | TCCCTATCAAACACCTGGTCA       | Minus           | 21     | 59710778  | 59710758 | 58.37 | 47.62 | 5                    | 2                       |
|               |                                             | <b>Length</b>    | 163                         |                 |        |           |          |       |       |                      |                         |
| Xpo1          | exportin 1                                  | <b>F</b>         | ACTGAGTCAATTCAGCAGTGA<br>AA | Plus            | 23     | 23291745  | 23291767 | 58.55 | 39.13 | 6                    | 3                       |
|               |                                             | <b>R</b>         | CGCCAAATCAACACTGGGTG        | Minus           | 20     | 23292069  | 23292050 | 60.04 | 55    | 3                    | 3                       |
|               |                                             | <b>Length</b>    | 325                         |                 |        |           |          |       |       |                      |                         |
| Cox5a         | cytochrome c oxidase subunit Va             | <b>F</b>         | CCACAGCAACGGTGTGAAAT        | Plus            | 20     | 57531862  | 57531881 | 59.33 | 50    | 5                    | 2                       |
|               |                                             | <b>R</b>         | CTGAGATGAAGCCCTCGGAC        | Minus           | 20     | 57531944  | 57531925 | 59.9  | 60    | 3                    | 2                       |
|               |                                             | <b>Length</b>    | 83                          |                 |        |           |          |       |       |                      |                         |
| Gaph          | glyceraldehyde-3-phosphate dehydrogenase    | <b>F</b>         | AGCTACGTGCACCCGTAAAG        | Plus            | 20     | 125165723 | 1.25E+08 | 60.39 | 55    | 6                    | 1                       |
|               |                                             | <b>R</b>         | CCCTATCAGTTCGGAGCCCA        | Minus           | 20     | 125165847 | 1.25E+08 | 61.05 | 60    | 3                    | 2                       |
|               |                                             | <b>Length</b>    | 125                         |                 |        |           |          |       |       |                      |                         |

**Forward primer – F; Reverse primer – R**

To complete the q-PCR experiment; total RNA was isolated of cell pellet, using Qiagen RNA isolation kit;