Supplemental Digital Content for AIDS-D-18-00814

Intranasal insulin therapy reverses hippocampal synaptodendritic injury and chronic cognitive impairment in EcoHIV-infected mice

Running Title: Intranasal insulin reverses HIV-NCI in mice

Boe-Hyun KIM1, Jennifer KELSCHENBACH1,Alejandra BORJABAD1,Eran HADAS1, Hongxia HE1, Mary Jane POTASH1, Michael T. NEDELCOVYCH2,3, Rana RAIS2,3,Norman J. HAUGHEY3, Justin C. MCARTHUR3, Barbara S. SLUSHER2,3#, David J. VOLSKY1\*#

1Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY 10029

2Johns Hopkins Drug Discovery Program and 3Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD 21205

\*Corresponding author: David J. Volsky (David.Volsky@mssm.edu)

#Correspondence and reprint requests: DavidJ. Volsky (David.Volsky@mssm.edu)

Barbara S. Slusher (bslusher@jhmi.edu)

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**SDC Figure Legend. Effect of insulin on EcoHIV infection in primary mouse macrophages in culture.** Mouse bone marrow-derived macrophages (BMM) were obtained by differentiation of C57BL/6J bone marrow cells in 24- or 6-well culture plates in RPMI medium supplemented with 10% FBS and 20% L929 cell-supernatant as a source of M-CSF [1]. After reaching about 80% confluence, nonadherent cells were removed, medium was changed to RPMI/10% FCS without L929 supernatant, and the resulting cultures of adherent cells, which contained >98% of F4/80-positive macrophages in flow cytometric verification, were used for experiments. (a) Insulin prophylaxis. BMM were supplemented with human insulin at the indicated doses in duplicates 1 h before infection with EcoHIV-luc at 1 pg p24/cell, cultured for 3 days, and analyzed. EcoHIV infection is presented as light units/106 live cells. \**p* < 0.5. (b) Abacavir prophylaxis. BMM were supplemented with Abacavir (ABC, Abcam, Cambridge, MA) at the indicated doses in duplicates, cultured for 24 h, infected with EcoHIV-luc at 1 pg p24/cell, cultured for 3 days, and analyzed for luminescence in BioTek Synergy H4 Hybrid Reader. EcoHIV infection is presented as light units/mg total cellular protein/well. \**p* < 0.05. (c) Insulin treatment. BMM were infected with EcoHIV-luc as in (a) except that cells were cultured for 7 days, supplemented with insulin at the doses listed and evaluated for HIV infection after 3 days. \**p* < 0.5

**SDC Table Legend.** **Effect of insulin treatment on expression of neuronal function and metabolism-related genes in the brain.** Total brain RNA was isolated and reverse-transcribed to cDNA as previously described [2]. The cDNAs were evaluated on CustomTaqMan® Array 96-Well Plates (ThermoFisher Scientific) according to manufacturer’s instructions. Reactions were run in a ThermoFisher (Applied Biosystems) 7900 instrument. The 88 genes represented in the table were chosen according to their published functions in synaptic plasticity, neuronal function, and brain metabolism. FC denotes fold change in gene expression relative to control (PBS i.p. + PBS IN); Significance was assessed with Student t-test p *<* 0.05.

Ten significantly downregulated genes, CAMK2A, NRGN, DLG4, NR4A1, GPRIN1, KALRN, NGFR, BDNF, MAPK14, and GRIA2 are implicated in synaptic plasticity, dendrite biology, and neuronal signal transmission, two in neurogenesis (DCX and NAV3), and two (PPAT and TMG5) in glutamine and energy metabolism (Fig. 3c).

Functions of these selected genes are: CAMK2A (Calcium/Calmodulin Dependent Protein Kinase II Alpha) encodes calcium signaling protein which is crucial for plasticity at glutamatergic synapses. This protein is composed of alpha, beta, gamma, and delta. The alpha unit is required for hippocampal long-term potentiation (LTP) and learning memories [3, 4]. NRGN (Neurogranin) is a calmodulin-binding protein expressed primarily in the brain, particularly in dendritic spines and is the major postsynaptic protein regulating the availability of calmodulin in the absence of Ca2+ [5]. DLG4 (Discs Large Homolog 4) or PSD95 (Postsynaptic Density Protein 95) is also known as SAP90 (Synapse-associated Protein 90) [6, 7]. DLG4 is exclusively located in the post synapse of neurons [8] and is involved in anchoring synaptic proteins. Direct and indirect binding partners of DGL4 include NRGN, NMDA receptors, AMPA receptors, and potassium channels [9]. DGL4 plays an important role in synaptic plasticity and the stabilization of synaptic changes during LTP [10]. NR4A1 (Nuclear Receptor subfamily 4, group A, member 1) is orphan nuclear receptor and may inhibit NF-kappa-B transactivation of IL2 [11]. Participates in energy homeostasis in the nucleus [12]. GPRIN1 (G Protein Regulated Inducer of Neurite Outgrowth 1) modulated neuronal signal transduction and affects learning behavior [13]. KALRN encodes Kalirin protein which was identified to interact with huntingtin-associated protein 1 [14] and also is known to play an important role in nerve growth and axonal development [15]. NGFR (Nerve Growth Factor Receptor) protein regulated p53 via negative feedback mechanism and plays as a novel p53 inactivator [16]. BDNF (Brain-Derived Neurotrophic Factor) is a widely expressed in cells and involved in many types of synaptic plasticity mediating learning and memory including emotional learning [17]. MAPK14 (Mitogen-activated protein kinase 14) is also called p38-alpha. P38α MAPK is implicated in cell survival/apoptosis, proliferation, differentiation, migration, mRNA stability and inflammatory response in different cell types through variety of target molecules [18]. GRIA2 (Glutamate Ionotropic Receptor AMPA type subunit 2) is one of the glutamate receptor. Glutamate receptors are the predominant excitatory neurotransmitter receptors in the mammalian brain and are activated in a variety of normal neurophysiologic processes [19].

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| Kim *et al.,* Supplemetal Digital Content Table |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Gene code | Gene description |  | PBS+Ins |  | EcoHIV |  | EcoHIV+Ins |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  | FC | ttest |  | FC | ttest | (FC) | FC | ttest |  |  |  |  |  |  |  |  |  |  |  |
| ADCY1 | adenylate cyclase 1 |  | 1.333 | 0.061 |  | 0.532 | 0.093 |  | 0.975 | 0.435 |  |  |  |  |  |  |  |  |  |  |  |
| ADCY8 | adenylate cyclase 8 |  | 1.125 | 0.280 |  | 0.511 | 0.063 |  | 1.140 | 0.241 |  |  | 88 genes on QPCR array |  |  |  |  |  |  |
| CAMK2A | calcium/calmodulin-dependent protein kinase II alpha |  | 1.200 | 0.195 |  | 0.407 | 0.030 | 2.100 | 0.933 | 0.325 |  |   | 14 genes significantly suppressed in murine HIV-NCI, p < 0.05, and normalized by insulin |
| CNR1 | cannabinoid receptor 1  |  | 1.141 | 0.259 |  | 0.400 | 0.066 |  | 0.892 | 0.257 |  |   | 40 genes trending suppressed in murine HIV-NCI, *p* < 0.1, and normalized by insulin |
| GRIA1 | glutamate receptor, ionotropic, AMPA1 (alpha 1) |  | 0.967 | 0.421 |  | 0.301 | 0.079 |  | 1.013 | 0.469 |  |  |  |  |  |  |  |  |  |  |  |
| GRIA2 | glutamate receptor, ionotropic, AMPA2 (alpha 2) |  | 0.967 | 0.444 |  | 0.265 | 0.039 | 3.800 | 1.017 | 0.470 |  |  |  |  |  |  |  |  |  |  |  |
| GPRIN1 | G protein-regulated inducer of neurite outgrowth 1 |  | 1.048 | 0.432 |  | 0.625 | 0.030 | 1.600 | 1.052 | 0.435 |  |  |  |  |  |  |  |  |  |  |  |
| GRIN2A | glutamate receptor, ionotropic, NMDA2A (epsilon 1) |  | 1.202 | 0.177 |  | 0.565 | 0.053 |  | 1.014 | 0.462 |  |  |  |  |  |  |  |  |  |  |  |
| GRIN2B | glutamate receptor, ionotropic, NMDA2B (epsilon 2) |  | 1.323 | 0.213 |  | 0.711 | 0.242 |  | 1.034 | 0.376 |  |  |  |  |  |  |  |  |  |  |  |
| GRIN2C | glutamate receptor, ionotropic, NMDA2C (epsilon 3) |  | 1.622 | 0.077 |  | 0.635 | 0.086 |  | 1.080 | 0.376 |  |  |  |  |  |  |  |  |  |  |  |
| MAPK1 | mitogen-activated protein kinase 1 |  | 1.008 | 0.468 |  | 0.384 | 0.109 |  | 0.962 | 0.375 |  |  |  |  |  |  |  |  |  |  |  |
| NTRK2 | neurotrophic tyrosine kinase, receptor, type 2 |  | 1.155 | 0.112 |  | 0.280 | 0.113 |  | 1.035 | 0.395 |  |  |  |  |  |  |  |  |  |  |  |
| PPP1CA | protein phosphatase 1, catalytic subunit, alpha isoform |  | 0.943 | 0.371 |  | 0.461 | 0.129 |  | 0.968 | 0.417 |  |  |  |  |  |  |  |  |  |  |  |
| PRKCA | protein kinase C, alpha |  | 1.036 | 0.388 |  | 0.536 | 0.092 |  | 1.066 | 0.306 |  |  |  |  |  |  |  |  |  |  |  |
| RAB3IP | RAB3A interacting protein |  | 1.079 | 0.321 |  | 0.609 | 0.073 |  | 0.966 | 0.361 |  |  |  |  |  |  |  |  |  |  |  |
| GRIA3 | glutamate receptor, ionotropic, AMPA3 (alpha 3) |  | 0.916 | 0.250 |  | 0.418 | 0.051 | 2.400 | 1.131 | 0.240 |  |  |  |  |  |  |  |  |  |  |  |
| GRIA4 | glutamate receptor, ionotropic, AMPA4 (alpha 4) |  | 1.088 | 0.338 |  | 0.362 | 0.102 |  | 1.145 | 0.268 |  |  |  |  |  |  |  |  |  |  |  |
| GRM1 | glutamate receptor, metabotropic 1 |  | 1.230 | 0.170 |  | 0.515 | 0.061 |  | 1.031 | 0.441 |  |  |  |  |  |  |  |  |  |  |  |
| GRM2 | glutamate receptor, metabotropic 2 |  | 1.083 | 0.240 |  | 0.660 | 0.064 |  | 0.938 | 0.224 |  |  |  |  |  |  |  |  |  |  |  |
| GRM5 | glutamate receptor, metabotropic 5 |  | 1.073 | 0.327 |  | 0.442 | 0.058 |  | 1.024 | 0.446 |  |  |  |  |  |  |  |  |  |  |  |
| NGFR | nerve growth factor receptor (TNFR superfamily, member 16) | 0.887 | 0.101 |  | 0.543 | 0.023 | 1.800 | 0.866 | 0.186 |  |  |  |  |  |  |  |  |  |  |  |
| PPP1R12A | protein phosphatase 1, regulatory (inhibitor) subunit 12A |  | 1.233 | 0.186 |  | 0.435 | 0.088 |  | 1.083 | 0.355 |  |  |  |  |  |  |  |  |  |  |  |
| PPP2CA | protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform | 1.043 | 0.367 |  | 0.157 | 0.134 |  | 1.156 | 0.163 |  |  |  |  |  |  |  |  |  |  |  |
| ADAM10 | a disintegrin and metallopeptidase domain 10 |  | 1.091 | 0.197 |  | 0.475 | 0.099 |  | 0.358 | 0.197 |  |  |  |  |  |  |  |  |  |  |  |
| DLG4 | discs, large homolog 4  |  | 1.090 | 0.178 |  | 0.442 | 0.050 | 2.400 | 0.940 | 0.263 |  |  |  |  |  |  |  |  |  |  |  |
| HOMER1 | homer homolog 1 |  | 1.154 | 0.173 |  | 0.433 | 0.063 |  | 0.990 | 0.470 |  |  |  |  |  |  |  |  |  |  |  |
| SYNPO | synaptopodin |  | 1.074 | 0.181 |  | 0.631 | 0.145 |  | 1.088 | 0.108 |  |  |  |  |  |  |  |  |  |  |  |
| BDNF | brain derived neurotrophic factor |  | 1.286 | 0.129 |  | 0.471 | 0.039 | 2.100 | 1.106 | 0.322 |  |  |  |  |  |  |  |  |  |  |  |
| DLG4 | discs, large homolog 4  |  | 1.139 | 0.105 |  | 0.501 | 0.064 |  | 0.886 | 0.118 |  |  |  |  |  |  |  |  |  |  |  |
| FGF2 | fibroblast growth factor 2 |  | 1.106 | 0.263 |  | 0.641 | 0.087 |  | 1.124 | 0.229 |  |  |  |  |  |  |  |  |  |  |  |
| ACHE | acetylcholinesterase |  | 0.990 | 0.383 |  | 0.517 | 0.080 |  | 0.991 | 0.453 |  |  |  |  |  |  |  |  |  |  |  |
| NRCAM | neuron-glia-CAM-related cell adhesion molecule |  | 1.101 | 0.276 |  | 0.325 | 0.105 |  | 1.195 | 0.148 |  |  |  |  |  |  |  |  |  |  |  |
| POU4F1 | POU domain, class 4, transcription factor 1 |  | 0.994 | 0.482 |  | 0.600 | 0.124 |  | 1.195 | 0.069 |  |  |  |  |  |  |  |  |  |  |  |
| APBB1 | amyloid beta (A4) precursor protein-binding, family B, member 1 | 0.681 | 0.163 |  | 0.517 | 0.149 |  | 1.036 | 0.466 |  |  |  |  |  |  |  |  |  |  |  |
| DCX | doublecortin |  | 1.072 | 0.311 |  | 0.539 | 0.016 | 1.900 | 0.933 | 0.263 |  |  |  |  |  |  |  |  |  |  |  |
| MAP2 | microtubule associated protein 2 |  | 0.995 | 0.484 |  | 0.368 | 0.098 |  | 1.056 | 0.369 |  |  |  |  |  |  |  |  |  |  |  |
| PARD3 | par-3 (partitioning defective 3)  |  | 1.541 | 0.027 |  | 0.747 | 0.186 |  | 0.939 | 0.322 |  |  |  |  |  |  |  |  |  |  |  |
| CDK5R1 | cyclin-dependent kinase 5, regulatory subunit 1 (p35) |  | 1.201 | 0.164 |  | 0.560 | 0.059 |  | 0.843 | 0.150 |  |  |  |  |  |  |  |  |  |  |  |
| NDN | necdin |  | 0.825 | 0.072 |  | 0.440 | 0.191 |  | 1.102 | 0.232 |  |  |  |  |  |  |  |  |  |  |  |
| ROBO1 | roundabout homolog 1  |  | 0.933 | 0.275 |  | 0.536 | 0.072 |  | 1.131 | 0.193 |  |  |  |  |  |  |  |  |  |  |  |
| EPHB1 | Eph receptor B1 |  | 1.144 | 0.281 |  | 0.589 | 0.081 |  | 0.947 | 0.311 |  |  |  |  |  |  |  |  |  |  |  |
| NR4A1 | nuclear receptor subfamily 4, group A, member 1 |  | 1.249 | 0.202 |  | 0.393 | 0.029 | 2.500 | 1.011 | 0.457 |  |  |  |  |  |  |  |  |  |  |  |
| PTGS2 | prostaglandin-endoperoxide synthase 2 |  | 1.130 | 0.173 |  | 0.722 | 0.237 |  | 0.953 | 0.423 |  |  |  |  |  |  |  |  |  |  |  |
| SYN2 | synapsin II |  | 1.181 | 0.171 |  | 0.361 | 0.093 |  | 1.044 | 0.383 |  |  |  |  |  |  |  |  |  |  |  |
| PCLO | piccolo (presynaptic cytomatrix protein) |  | 1.218 | 0.099 |  | 0.458 | 0.082 |  | 1.066 | 0.319 |  |  |  |  |  |  |  |  |  |  |  |
| STX1A | syntaxin 1A  |  | 0.960 | 0.307 |  | 0.602 | 0.061 |  | 1.058 | 0.259 |  |  |  |  |  |  |  |  |  |  |  |
| RIMS1 | regulating synaptic membrane exocytosis 1 |  | 1.408 | 0.073 |  | 0.472 | 0.102 |  | 1.009 | 0.481 |  |  |  |  |  |  |  |  |  |  |  |
| KCNMA1 | potassium large conductance calcium-activated channel, subfamily M, alpha member 1 | 1.234 | 0.142 |  | 0.417 | 0.054 |  | 1.023 | 0.451 |  |  |  |  |  |  |  |  |  |  |  |
| KCNH7 | potassium voltage-gated channel, subfamily H (eag-related), member 7 | 1.120 | 0.189 |  | 0.659 | 0.160 |  | 1.108 | 0.231 |  |  |  |  |  |  |  |  |  |  |  |
| NLGN1 | neuroligin 1 |  | 1.244 | 0.129 |  | 0.671 | 0.232 |  | 1.009 | 0.476 |  |  |  |  |  |  |  |  |  |  |  |
| NRGN | neurogranin |  | 0.849 | 0.077 |  | 0.516 | 0.033 | 1.900 | 1.017 | 0.402 |  |  |  |  |  |  |  |  |  |  |  |
| SLC1A2/EAAT2 | excitatory amino acid transporter 2 |  | 1.261 | 0.209 |  | 0.182 | 0.127 |  | 0.944 | 0.420 |  |  |  |  |  |  |  |  |  |  |  |
| RYR2 | ryanodine receptor 2, cardiac |  | 1.176 | 0.170 |  | 0.483 | 0.086 |  | 1.207 | 0.098 |  |  |  |  |  |  |  |  |  |  |  |
| CAMK1D | Calcium/calmodulin-dependent protein kinase ID |  | 1.300 | 0.224 |  | 0.691 | 0.297 |  | 1.058 | 0.429 |  |  |  |  |  |  |  |  |  |  |  |
| CAMK4 | calcium/calmodulin-dependent protein kinase IV |  | 1.285 | 0.146 |  | 0.480 | 0.096 |  | 1.081 | 0.350 |  |  |  |  |  |  |  |  |  |  |  |
| EPHA5 | Eph receptor A5 |  | 1.156 | 0.216 |  | 0.554 | 0.087 |  | 0.970 | 0.415 |  |  |  |  |  |  |  |  |  |  |  |
| EPHA7 | Eph receptor A7 |  | 1.020 | 0.428 |  | 0.620 | 0.077 |  | 1.081 | 0.132 |  |  |  |  |  |  |  |  |  |  |  |
| KALRN | kalirin, RhoGEF kinase |  | 1.238 | 0.167 |  | 0.614 | 0.046 | 1.600 | 1.071 | 0.352 |  |  |  |  |  |  |  |  |  |  |  |
| NEGR1 | neuronal growth regulator 1 |  | 1.263 | 0.132 |  | 0.502 | 0.073 |  | 1.253 | 0.082 |  |  |  |  |  |  |  |  |  |  |  |
| NAV3 | neuron navigator 3 |  | 1.096 | 0.271 |  | 0.626 | 0.049 | 1.600 | 1.081 | 0.278 |  |  |  |  |  |  |  |  |  |  |  |
| ASS1 | Argininosuccinate synthetase 1 |  | 0.979 | 0.448 |  | 0.526 | 0.081 |  | 1.284 | 0.094 |  |  |  |  |  |  |  |  |  |  |  |
| CAD | carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase | 0.963 | 0.363 |  | 0.696 | 0.089 |  | 1.066 | 0.119 |  |  |  |  |  |  |  |  |  |  |  |
| CPS1 | carbamoyl-phosphate synthetase 1 |  | 1.192 | 0.117 |  | 0.655 | 0.129 |  | 0.635 | 0.177 |  |  |  |  |  |  |  |  |  |  |  |
| GFPT1 | glutamine--fructose-6-phosphate transaminase 1 |  | 1.105 | 0.260 |  | 0.544 | 0.110 |  | 1.082 | 0.324 |  |  |  |  |  |  |  |  |  |  |  |
| GLS | glutaminase |  | 1.102 | 0.329 |  | 0.325 | 0.087 |  | 1.123 | 0.284 |  |  |  |  |  |  |  |  |  |  |  |
| GLUD1 | glutamate dehydrogenase 1 |  | 1.052 | 0.369 |  | 0.222 | 0.115 |  | 1.204 | 0.130 |  |  |  |  |  |  |  |  |  |  |  |
| GOT2 | glutamate oxaloacetate transaminase 2, mitochondrial |  | 1.072 | 0.282 |  | 0.351 | 0.123 |  | 1.050 | 0.331 |  |  |  |  |  |  |  |  |  |  |  |
| NIT2 | nitrilase family, member 2 |  | 1.043 | 0.223 |  | 0.654 | 0.100 |  | 1.144 | 0.072 |  |  |  |  |  |  |  |  |  |  |  |
| PPAT | phosphoribosyl pyrophosphate amidotransferase |  | 1.046 | 0.387 |  | 0.627 | 0.048 | 1.600 | 1.254 | 0.117 |  |  |  |  |  |  |  |  |  |  |  |
| GLB1 | galactosidase, beta 1 |  | 0.992 | 0.467 |  | 0.649 | 0.116 |  | 1.237 | 0.031 |  |  |  |  |  |  |  |  |  |  |  |
| GOT1 | glutamate oxaloacetate transaminase 1, soluble |  | 1.577 | 0.205 |  | 0.415 | 0.250 |  | 1.101 | 0.008 |  |  |  |  |  |  |  |  |  |  |  |
| GCLM | glutamate-cysteine ligase, modifier subunit |  | 1.155 | 0.207 |  | 0.389 | 0.095 |  | 1.125 | 0.247 |  |  |  |  |  |  |  |  |  |  |  |
| GFPT1 | glutamine fructose-6-phosphate transaminase 1 |  | 1.020 | 0.451 |  | 0.525 | 0.088 |  | 1.049 | 0.413 |  |  |  |  |  |  |  |  |  |  |  |
| QSER1 | glutamine and serine rich 1 |  | 1.202 | 0.102 |  | 0.585 | 0.126 |  | 1.050 | 0.384 |  |  |  |  |  |  |  |  |  |  |  |
| TGM2 | transglutaminase 2, C polypeptide |  | 1.353 | 0.104 |  | 0.799 | 0.149 |  | 1.000 | 0.499 |  |  |  |  |  |  |  |  |  |  |  |
| TGM5 | transglutaminase 5 |  | 1.170 | 0.206 |  | 0.665 | 0.049 | 1.500 | 1.413 | 0.078 |  |  |  |  |  |  |  |  |  |  |  |
| SOD2 | superoxide dismutase 2 |  | 1.074 | 0.373 |  | 0.368 | 0.097 |  | 1.173 | 0.253 |  |  |  |  |  |  |  |  |  |  |  |
| UCP2 | Uncoupling protein 2  |  | 1.115 | 0.182 |  | 0.489 | 0.076 |  | 1.039 | 0.332 |  |  |  |  |  |  |  |  |  |  |  |
| AKT3 | thymoma viral proto-oncogene 3 |  | 1.835 | 0.148 |  | 0.642 | 0.333 |  | 0.736 | 0.248 |  |  |  |  |  |  |  |  |  |  |  |
| PTEN | phosphatase and tensin homolog |  | 0.998 | 0.498 |  | 0.435 | 0.157 |  | 1.176 | 0.295 |  |  |  |  |  |  |  |  |  |  |  |
| PDK1 | pyruvate dehydrogenase kinase, isoenzyme 1 |  | 0.392 | 0.162 |  | 0.278 | 0.164 |  | 0.907 | 0.060 |  |  |  |  |  |  |  |  |  |  |  |
| MAPK10 | mitogen-activated protein kinase 10 |  | 1.010 | 0.462 |  | 0.372 | 0.097 |  | 1.091 | 0.238 |  |  |  |  |  |  |  |  |  |  |  |
| MAPK14 | mitogen-activated protein kinase 14 |  | 1.211 | 0.113 |  | 0.501 | 0.025 | 2.000 | 1.020 | 0.439 |  |  |  |  |  |  |  |  |  |  |  |
| HDAC2 | histone deacetylase 2 |  | 1.072 | 0.345 |  | 0.305 | 0.092 |  | 1.258 | 0.149 |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |