# Gene expression patterns associated with neurological disease in HIV infection

**Running title**: Patterns of gene dysregulation neuroAIDS

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**Abstract.**

To provide new insight into the pathogenesis of neurocognitive impairments (NCI) in HIV infection, we used the Gene Set Enrichment Analysis (GSEA) algorithm to analyze pathway dysregulations in gene expression profiles of HIV-infected patients with or without NCI and HIV encephalitis (HIVE). While HIVE was characterized by widespread inflammation and tissue damage, gene expression evidence of induction of interferon (IFN), cytokines and tissue injury was apparent in all brain regions studied before the emergence of NCI. Various degrees of white matter changes were present in all HIV-infected subjects and were the primary manifestation in patients with NCI in the absence of HIVE. The latter showed a distinct pattern of immune activation with induction of chemokines, cytokines, β-defensins, and limited IFN induction.

Altogether results indicate that significant neuroinflammation and neuronal suffering precede NCI. Patients with NCI without HIVE showed a predominantly white matter dysfunction with a distinct pattern of immune activation.

Keywords:

Neurodegeneration, pathogenesis, neuroAids, neuroinflammation.

Abbreviations:

Asymptomatic neurocognitive impairment (ANI)

Combination antiretroviral therapy (cART)

Gene Set Enrichment Analysis (GSEA)

HIV-associated neurological disease (HAND)

HIV encephalitis (HIVE)

Interferon (IFN)

NeuroAIDS Tissue Consortium (NNTC)

Neurocognitive impairments (NCI)

## Introduction

While the prevalence of severe HIV-associated dementia (HAD) has decreased since the introduction of combination antiretroviral therapy (cART), milder and chronic forms of neurocognitive impairment (NCI) including asymptomatic neurocognitive impairment (ANI) and HIV-associated neurocognitive disorders (HAND) as well as HIV-associated major depressive disorder remain high (1-7). HIV encephalitis (HIVE) is considered to be the main neuropathological substrate of HAD (8-10). NCI in the setting of cART is associated with synaptodendritic degeneration (7, 11, 12). While the brain represents a sanctuary where HIV can persist due to suboptimal penetration of antiretroviral drugs (13), various studies highlighted the occurrence of NCI even in the setting of viral suppression (14, 15). Chronic neuroinflammation is believed to drive neurodegeneration in cART-era HAND (7, 9, 16, 17). However, the pathogenic mechanisms behind HAND remain unclear.

To identify gene expression correlates of neurological disease progression in HIV, we analyzed pathway dysregulations in brain regions of patients in the National NeuroAIDS Tissue Consortium (NNTC) gene expression profile dataset. The NNTC dataset consists of samples from 3 different brain regions (white matter, basal ganglia, prefrontal cortex) of control and HIV-infected patients with or without NCI and HIVE (18). For pathway analysis we used the Gene Set Enrichment Analysis (GSEA), a computational method to assess whether a priori defined sets of genes show statistically significant differences between biological states (19). GSEA was used in

77 conjunction with gene sets from the Molecular Signatures Database (MSigDb), including

78 canonical pathways in the C2 collection (20).

## Materials and Methods.

Dataset and analysis. Clinical and demographic features of the subjects in the NNTC gene expression dataset used for the study are shown in Supplementary Table 1. Raw data were downloaded from GEO (GSE35864). We filtered out 9 samples based on quality controls (actin3/actin5 ratio, gapdh3/gapdh5 ratio, NUSE (Normalized Unscaled Standard Errors) and RLE (Relative Log Expression) computed with the packages simpleaffy and affyPLM in R). Normalization was done using gcrma (21). We further checked the expression of markers of neurons (RBFOX3) and olygodendrocytes (MBP) to validate the brain region profiled for white matter and prefrontal cortex samples and further excluded 2 samples that had conflicting expression according to their classification (D1 WM and D1 FC).

Pathway analysis. For pathway analysis, we selected one representative probe per gene based on the highest observed coefficient of variation of the probes across the samples. The dataset was interrogated for pathway enrichment using the GSEA algorithm and the canonical pathways from the MSidDb C2 collection (1,237 pathways with at least 10 genes). GSEA was run using 1,000 shuffling of the reference list. Significance was assessed using the False Discovery Rate (FDR) computed as defined in the original GSEA publication for controlling the number of false positives in each GSEA analysis (19). Differential expression was computed using a Welch t-test from the package Class Comparison in R 3.3.1. We defined the pathways commonly differentially regulated in each comparison as the pathways satisfying an FDR < 0.01 in at least 2 of the 3 brain regions for that comparison while pathways specific to one

region were defined as pathways satisfying FDR < 0.01 in that region and FDR > 0.25 in the other 2 regions.

Pathways activity: The activity of a pathway in a sample was computed the following way: we first z-transformed the gene expression profiles to normalize the expression of each gene across samples. We then computed the enrichment score (ES) of a gene set using this z-transformed matrix of expression, as described in the original description of GSEA (19). The ES corresponds to the relative activity of a gene set in a sample as compared to all others. Hence, the samples with the highest ES are the samples with the highest relative expression of the genes belonging to this set among the samples belonging to the gene expression matrix.

GSEA: Gene set enrichment analysis was implemented in R and follows the method described in (19). Null distribution was obtained by 1,000 shuffling of the reference list. Gene signatures were obtained by ranking the genes according to the sign of the statistics (S) and the p-value (p) of the test with the following metric: -1×sign(S)×log(P,10).

## Results

**Pathway analysis of the NNTC dataset.** The NNTC dataset was interrogated for pathway enrichment using the canonical pathways from the MSigDb C2 collection and the GSEA algorithm (19) (See methods). We compared the following four groups: A: controls**;** B: HIV-infected no NCI no HIVE**;** C: HIV-infected with NCI no HIVE**;** D: HIV-infected with NCI and HIVE (Fig. 1). All results are presented in supplementary tables in Supplementary Tables 2-6.

**Identification of pathways dysregulated in HIV-infected patients without NCI *vs.* uninfected controls (B-A comparison).** We identified 24 pathways concordantly differentially regulated in at least 2 brain regions in this transition (Table 1). Genes driving the enrichment (on the left of the leading edge corresponding to the peak of the running enrichment in GSEA as shown in Fig. 2A) were retrieved for each region (Fig. 3). These pathways and genes indicate a significant activation of IFN and cytokine signaling prior to the onset of NCI. Both genes regulated by type I and type II IFN were activated in HIV infection without NCI (Fig. 2, 3). IFN-regulated genes, such as MCH class I genes, were induced in all brain regions of HIV-infected patients without NCI as compared to uninfected controls (Fig. 2, 3).

We then looked at pathways specifically differentially regulated in one brain region as compared to the two other regions. To this end, we selected pathways enriched at FDR < 0.01 in one region and FDR > 0.25 in the other two. We observed 6 pathways meeting the criteria in the white matter, 4 in the prefrontal cortex and 2 in the basal ganglia.

Pathways enriched in the white matter in the B-A comparison are indicative of immune activation, and complement induction (e.g., BIOCARTA COMP PATHWAY, BIOCARTA CTL PATHWAY, REACTOME IMMUNOREGULATORY INTERACTIONS BETWEEN A LYMPHOID AND A NON LYMPHOID CELL). We also observed increased expression of calpain-related genes (BIOCARTA UCALPAIN PATHWAY) in the prefrontal cortex and calpain-related and caspases-related genes in the basal ganglia (KEGG APOPTOSIS) as well as evidence of activation of the apoptosis-mediating p75 receptor (PID P75 NTR PATHWAY) and TNF-$\alpha$ signaling (PID TNF PATHWAY) in both prefrontal cortex and basal ganglia, indicative of tissue damage. Downregulation of genes related to neurotransmission was also evident in the prefrontal cortex and basal ganglia of patients with HIV but no NCI (group B) compared to control subjects (e.g., REACTOME LIGAND GATED ION CHANNEL TRANSPORT, KEGG NEUROACTIVE LIGAND RECEPTOR INTERACTION, Fig. 4), (Supplementary Table 2).

**Identification of pathways differentially regulated in HIV-infected patients with NCI without HIVE vs. uninfected controls (C-A comparison).**

HIV-infected patients with NCI and no HIVE (group C), showed significant changes specific to the white matter compared to uninfected controls. Upregulated pathways are indicative of immune activation involving chemokine, cytokines and $\beta$-defensins induction (e.g., REACTOME CHEMOKINE RECEPTORS BIND CHEMOKINES; KEGG CYTOKINE CYTOKINE RECEPTOR INTERACTION, REACTOME BETA DEFENSINS, KEGG AUTOIMMUNE THYROID DISEASE, KEGG ALLOGRAFT REJECTION), oxidative stress and cytochrome P450 enzymes (KEGG DRUG METABOLISM CYTOCHROME P450, REACTOME BIOLOGICAL OXIDATIONS), matrix

metalloproteases (MMPs) (NABA MATRISOME ASSOCIATED), and downregulation of genes related to RNA transcription and processing (e.g., REACTOME RNA POL II TRANSCRIPTION, KEGG SPLICEOSOME), (Supplementary Table 3), Fig. 5.

**Identification of pathways differentially regulated between HIV-infected patients with NCI and no HIVE vs. HIV-infected without NCI (C-B comparison).**

Two pathways were concordantly differentially regulated between groups B and C in all brain regions. These pathways are indicative of type I IFN activation in HIV-infected patients without NCI (group B), as indicated above. We also identified 47 pathways specifically differentially regulated in the prefrontal cortex. Among the pathways upregulated in group B as compared to C in the prefrontal cortex, were pathways indicative of tissue damage (e.g., REACTOME REGULATION OF APOPTOSIS), RNA transcription and processing (e.g., REACTOME METABOLISM OF RNA, KEGG RIBOSOME), and pathways related to protein degradation (e.g., KEGG PROTEASOME, REACTOME AUTODEGRADATION OF THE E3 UBIQUITIN LIGASE COP1, REACTOME APC C CDC20 MEDIATED DEGRADATION OF MITOTIC PROTEINS), (Supplementary Table 4).

**Identification of pathways differentially regulated between HIV-infected patients with HIVE vs. patients without NCI (D-B comparison).**

Pathways dysregulated in all brain regions in HIVE (group D) as compared to patients with HIV without NCI (group B) are suggestive of disruption of protein folding, a mechanism of neurodegeneration (e.g., REACTOME POST CHAPERONIN TUBULIN FOLDING PATHWAY, REACTOME PREFOLDIN MEDIATED TRANSFER OF

SUBSTRATE TO CCT TRIC). Other broadly dysregulated pathways in patients with HIVE (group D) as compared to patients with HIV without NCI (group B) indicate greater and broader activation of inflammatory and immune activation genes in group D as compared to group B. Genes regulated by both type I and type II IFN that were found activated in HIV infection without NCI (group B) as compared to uninfected controls, generally showed greater activation in patients with HIVE (group D). For instance, REACTOME INTERFERON GAMMA SIGNALING was increased in group D in basal ganglia in comparison to group B, and REACTOME INTERFERON ALPHA BETA SIGNALING was increased in group D in both white matter and basal ganglia in comparison to group B. All pathways specifically upregulated in basal ganglia in group D were involved in immune activation (e.g., REACTOME NUCLEAR EVENTS KINASE AND TRANSCRIPTION FACTOR ACTIVATION, REACTOME CYTOKINE SIGNALING IN IMMUNE SYSTEM, KEGG LEISHMANIA INFECTION, PID TCR PATHWAY).

Interestingly, the white matter in group D did not show any specific differentially regulated pathways in comparison to group B; conversely, the prefrontal cortex had 121 pathways and basal ganglia had 16 pathways significantly activated in group D in comparison to group B. Among the pathways upregulated in the prefrontal cortex in group D were pathways indicative of production of cytokine, chemokines and β-defensins (e.g., KEGG CYTOKINE CYTOKINE RECEPTOR INTERACTION, REACTOME CHEMOKINE RECEPTORS BIND CHEMOKINES, REACTOME BETA DEFENSINS). Pathways indicative of neurodegeneration were differentially regulated between D and B in frontal cortex including KEGG PARKINSONS DISEASE and KEGG HUNTINGTONS DISEASE (Fig. 6). These pathways include genes indicative of trophic

interaction, protein misfolding and mitochondrial function. We also identified downregulated pathways related to mitochondria and energy metabolism were decreased in group D in all brain regions at FDR < 0.2 (e.g., REACTOME TCA CYCLE AND RESPIRATORY ELECTRON TRANSPORT, REACTOME PYRUVATE METABOLISM AND CITRIC ACID TCA CYCLE, REACTOME GLYCOLYSIS), (Supplementary Table 5).

**Identification of pathways differentially regulated between HIV-infected patients with HIVE vs. patients with NCI and no HIVE (D-C comparison).**

We identified 27 pathways concordantly differentially regulated at the C to D comparison. Seventeen pathways were upregulated in group D (HIVE) as compared to group C (NCI without HIVE) and largely reflected activation in HIVE of IFN response (e.g., REACTOME INTERFERON SIGNALING, REACTOME INTERFERON GAMMA SIGNALING, KEGG ANTIGEN PROCESSING AND PRESENTATION), immune activation and inflammatory cytokine signaling (e.g., REACTOME CYTOKINE SIGNALING IN IMMUNE SYSTEM, KEGG CYTOKINE CYTOKINE RECEPTOR INTERACTION, REACTOME INNATE IMMUNE SYSTEM), apoptosis (BIOCARTA DEATH PATHWAY), protein misfolding (REACTOME PREFOLDIN MEDIATED TRANSFER OF SUBSTRATE TO CCT TRIC), and HIV infection (PID HIV NEF PATHWAY, REACTOME LATE PHASE OF HIV LIFE CYCLE).

Ten pathways were downregulated in group D and included pathways related to translation and transcription, as seen in the A to B transition, likely reflecting transcriptional/translational dysregulations brought about by IFN activation (e.g.,

REACTOME TRANSPORT OF RIBONUCLEOPROTEINS INTO THE HOST NUCLEUS), HIV expression (REACTOME INTERACTIONS OF VPR WITH HOST CELLULAR PROTEINS, REACTOME LATE PHASE OF HIV LIFE CYCLE), impaired neuronal communication (REACTOME TRANSMISSION ACROSS CHEMICAL SYNAPSES), and energy metabolism (REACTOME CITRIC ACID CYCLE TCA CYCLE, REACTOME PYRUVATE METABOLISM AND CITRIC ACID TCA CYCLE).

Pathways related to neurodegenerative/neuronal pathways were differentially regulated in the prefrontal cortex and basal ganglia in patients with HIVE, including KEGG HUNTINGTONS DISEASE, KEGG PARKINSONS DISEASE, REACTOME NEURONAL SYSTEM (Fig. 6). A significant component of these pathways are genes involved in mitochondria function and energy metabolism. No pathways were specifically different in white matter between groups C and D, while 37 pathways were specific to the prefrontal cortex and 11 to basal ganglia. Several prefrontal cortex-specific pathways were downregulated in group D and included cell cycle regulation while basal ganglia pathways were related to translation/transcription and immune regulation (BIOCARTA D4GDI PATHWAY, BIOCARTA 41BB PATHWAY, PID CD8 TCR DOWNSTREAM PATHWAY, PID IL12 STAT4 PATHWAY), (Supplementary Table 6).

## Discussion.

While dementia and HIV encephalitis are late consequences of HIV/AIDS, HIV enters the brain early after infection and remains in the brain throughout the course of infection. A considerable body of observations indicate that neuroinflammatory markers correlate with disease progression and the emergence of NCI in neuroAIDS (22-24). Proinflammatory cytokines and chemokines including IFN-$\alpha$, TNF-$\alpha$ and CCL2 that are secreted by astrocytes and microglia have long been implicated in the pathogenesis of neuroAIDS (25-29). For instance, IFN-$\alpha$ in the cerebrospinal fluid has been observed to be higher in HAD compared with HIV-infected patients without HAD (25, 28, 30). Here, we show that HIV infection is associated with substantial dysregulations of gene expression related to immune activation before the onset of NCI and HIVE.

A primary finding in the present study is that we observed gene expression evidence of IFN induction in patients with HIV infection without NCI (group B) as well as in patients with HIVE (group D). Induction of both IFN type I and type II responsive genes was seen in patients with HIV infection without NCI (group B) in all brain regions studied. Among the genes differentially regulated within these pathways were IFN-responsive genes such as HLA-A, -B, -C, -G, -F, adhesion molecules such as VCAM-1, and ISG15 and IFI6 (31-33).

Chronic IFN expression is considered a key contributor to inflammation in neuroAIDS as well as a potential cause of NCI and depression vulnerability. However, data on the contribution of IFN activation to NCI are conflicting. Mice with transgene

283 expression of IFN-α in astrocytes develop a dose-dependent inflammatory
284 encephalopathy (34). Yet IFN-α transgenic expression in the central nervous system
285 induced only mild effects in an egocentric spatial working memory test (35). However,
286 the latter may also reflect compensatory changes as passively administered IFN-β
287 impaired spatial memory in mice in another study (36). A recent study suggested a role
288 for IFN-γ in shaping fronto-cortical connections and social behavior (37), which is
289 consistent with a potential role of excessive IFN activation in the pathogenesis of NCI.
290 In a recent large multi-center trial, depression was not significantly increased by IFN-
291 β□treatment for multiple sclerosis (MS) (38). The early induction of IFN in patients of the
292 NNTC dataset (patients with HIV without NCI, group B) is reminiscent of previous
293 studies in which IFN induction was not closely correlated with NCI, e.g., (39), and raises
294 the possibilities that either protracted IFN dysregulation may be required to produce NCI
295 or that it may be a co-factor in NCI pathogenesis.
296
297 Also evident in HIV-infected patients without NCI was the activation of
298 mechanisms indicative of tissue injury, such as expression of matrix metalloproteases
299 (MMP) and complement-related genes in the white matter. MMP expression by HIV-1
300 infected monocytes and macrophages is recognized as a pathogenic mechanism in
301 neuroAIDS (40). Elevated MMP levels can contribute to microglial activation, infiltrate
302 through cleavage of adhesion molecules, neuronal and synaptic injury, as well as blood-
303 brain barrier disruption (41-44). MMP increases were present in the white matter in HIV-
304 infected patients with NCI and no HIVE. In patients with HIVE, induction of MMPs was
305 also evident in the prefrontal cortex and basal ganglia.

306 Another key finding in the study is that patients with NCI without HIVE (group C)
307 in the NNTC cohort did not show significant activation of IFN, unlike patients in groups B
308 and D. This discordant regulation of IFN signaling did not appear to be associated with
309 antiretroviral therapy as patients with NCI and no HIVE include both patients treated
310 with antiretrovirals and untreated patients. Conversely, patients with NCI without HIVE
311 (group C) had increased expression of chemokines, cytokines and β-defensins in the
312 white matter. Other pro-inflammatory markers were also concomitantly increased in the
313 white matter of patients with NCI and no HIVE. Evidence of chemokine and cytokine
314 expression were present in all HIV-infected groups in the study. β-defensins were
315 induced also in patients with HIVE.
316
317 Chemokines have been implicated in impairing cognition, Alzheimer's disease
318 and depression as well as other psychiatric conditions (45). Increased immunoreactivity
319 for MCP-2 was noted in MS lesions (46). A chemokine gene cluster has been
320 associated with age of onset of Alzheimer's (47). A higher level of CCL2 in CSF, and a
321 CCL2 -2578G allele, have been associated with worse neurocognitive functioning in HIV
322 (48). Animal studies, while scant, are consistent with a possible role for chemokines in
323 NCI. For instance, chemokine signaling was increased by SIV infection and
324 methamphetamine exposure in macaques (49, 50). Chemokines can induce changes
325 leading to impaired hippocampal synaptic transmission, plasticity and memory (50, 51).
326 Evidence also suggests a role for defensins in the chronic inflammation associated with
327 degenerative brain diseases, and in particular Alzheimer's disease (52, 53). Defensins-
328 related pathways were also induced in HIVE, but showed no consistent regulation in

329 HIV-infected patients with no NCI, suggesting a possible contribution to the
330 pathogenesis of NCI.
331
332 In HIV without NCI, genes related to neurotransmission were also downregulated
333 in the prefrontal cortex and basal ganglia while genes related to apoptosis, such as
334 calpain-related mechanisms, which contribute to neurodegeneration in HIV (54), were
335 induced in the basal ganglia and prefrontal cortex. Conversely, no pathways showed
336 significant dysregulations in the prefrontal cortex and basal ganglia in HIV patients with
337 NCI and no HIVE. In HIVE, multiple pathways indicative of impaired mitochondria and
338 energy metabolism were differentially regulated. In NCI without HIVE, we observed
339 increased expression of cytochrome P450 enzymes, which may indicate oxidative
340 stress (55).
341
342 The anatomical distribution of the gene expression programs dysregulated in the
343 NNTC dataset appears to reflect brain-region specific dynamics in neurological disease
344 progression in HIV/AIDS. In particular, we observed some degree of white matter
345 alteration of gene expression in all HIV-infected groups with and without NCI and HIVE.
346 However, gene expression changes in patients with NCI without HIVE (group C) were
347 localized to the white matter and had a specific gene expression profile. Lack of gene
348 expression changes suggestive of neuronal injury in the prefrontal cortex and basal
349 ganglia in patients with NCI without HIVE (group C) suggests that they may not be
350 accompanied by significant neuronal atrophy, but that white matter pathology likely
351 drives NCI in these patients. Prominent white matter gene expression changes were
352 also present in HIVE, which was also characterized by considerable gene expression

353 changes in the prefrontal cortex and basal ganglia. White matter damage correlating
354 with the severity of cognitive manifestations has been observed since the early days of
355 the HIV pandemic (8, 56). Evidence of white matter injury in HIV-infected patients with
356 and without NCI is also demonstrated in recent imaging studies (57, 58). In addition to
357 white matter changes, gene expression in HIVE was characterized by considerable
358 changes in the prefrontal cortex and basal ganglia. This is also in apparent agreement
359 with the association of NCI with progression of functional abnormalities involving the
360 basal ganglia and the prefrontal cortex as well as with generalized white matter damage
361 (56, 59-62).
362
363 The present study has several limitations. Primarily, the NNTC dataset groups
364 are of small sample size that was further reduced as part of the quality control analysis.
365 Larger studies will be needed to better understand the pathogenesis and progression of
366 neurological disease and to adequately represent all possible variants of central
367 nervous system disease. For instance, gene expression results of the group of HIV-
368 infected patients with NCI and without HIVE raise several questions, including if this is a
369 distinct nosologic variant of neuroAIDS or if it is a stage in the progression of HIV brain
370 disease.
371
372 In conclusion, in the present study we explored patterns of gene expression
373 dysregulation in patients in the NNTC neuroAIDS gene expression dataset. Results
374 point to gene expression changes indicative of immune activation characterized by IFN
375 and cytokine expression as well as evidence of neuronal suffering preceding NCI.
376 Interestingly, the group of HIV-infected patients with NCI without HIVE showed a

377 preeminently white matter dysfunction characterized by a distinct pattern of immune
378 activation with low IFN. Larger studies are necessary to better understand the
379 pathogenesis of neurological disease and its progression, to evaluate the impact of
380 therapy on various HIV disease conditions, and to identify better therapeutic targets and
381 strategies for NCI in HIV.

## Competing interests

The authors declare that they have no competing interests"

## Authors' contributions

PPS and CL designed study, analyzed data

PPS, VRC, EM, and CL interpreted results and wrote paper

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## References


1. **Heaton RK, Franklin DR, Ellis RJ, McCutchan JA, Letendre SL, Leblanc S, Corkran SH, Duarte NA, Clifford DB, Woods SP, Collier AC, Marra CM, Morgello S, Mindt MR, Taylor MJ, Marcotte TD, Atkinson JH, Wolfson T, Gelman BB, McArthur JC, Simpson DM, Abramson I, Gamst A, Fennema-Notestine C, Jernigan TL, Wong J, Grant I.** 2011. HIV-associated neurocognitive disorders before and during the era of combination antiretroviral therapy: differences in rates, nature, and predictors. J Neurovirol **17:**3-16.
2. **Cysique LA, Maruff P, Brew BJ.** 2004. Prevalence and pattern of neuropsychological impairment in human immunodeficiency virus-infected/acquired immunodeficiency syndrome (HIV/AIDS) patients across pre- and post-highly active antiretroviral therapy eras: a combined study of two cohorts. J Neurovirol **10:**350-357.
3. **Tozzi V, Balestra P, Serraino D, Bellagamba R, Corpolongo A, Piselli P, Lorenzini P, Visco-Comandini U, Vlassi C, Quartuccio ME, Giulianelli M, Noto P, Galgani S, Ippolito G, Antinori A, Narciso P.** 2005. Neurocognitive impairment and survival in a cohort of HIV-infected patients treated with HAART. AIDS Res Hum Retroviruses **21:**706-713.
4. **Neuenburg JK, Brodt HR, Herndier BG, Bickel M, Bacchetti P, Price RW, Grant RM, Schlote W.** 2002. HIV-related neuropathology, 1985 to 1999: rising prevalence of HIV encephalopathy in the era of highly active antiretroviral therapy. J Acquir Immune Defic Syndr **31:**171-177.
5. **McArthur JC.** 2004. HIV dementia: an evolving disease. J Neuroimmunol **157:**3-10.
6. **Brew BJ.** 2004. Evidence for a change in AIDS dementia complex in the era of highly active antiretroviral therapy and the possibility of new forms of AIDS dementia complex. Aids **18 Suppl 1:**S75-78.
7. **Everall I, Vaida F, Khanlou N, Lazzaretto D, Achim C, Letendre S, Moore D, Ellis R, Cherne M, Gelman B, Morgello S, Singer E, Grant I, Masliah E.** 2009. Cliniconeuropathologic correlates of human immunodeficiency virus in the era of antiretroviral therapy. J Neurovirol doi:914601275 [pii] 10.1080/13550280903131915**:**1-11.
8. **Everall IP, Hansen LA, Masliah E.** 2005. The shifting patterns of HIV encephalitis neuropathology. Neurotox Res **8:**51-61.
9. **Persidsky Y, Gendelman HE.** 2003. Mononuclear phagocyte immunity and the neuropathogenesis of HIV-1 infection. J Leukoc Biol **74:**691-701.
10. **McArthur JC, Hoover DR, Bacellar H, Miller EN, Cohen BA, Becker JT, Graham NM, McArthur JH, Selnes OA, Jacobson LP, et al.** 1993. Dementia in AIDS patients: incidence and risk factors. Multicenter AIDS Cohort Study. Neurology **43:**2245-2252.
11. **Ellis R, Langford D, Masliah E.** 2007. HIV and antiretroviral therapy in the brain: neuronal injury and repair. Nat Rev Neurosci **8:**33-44.
12. **Masliah E, Heaton RK, Marcotte TD, Ellis RJ, Wiley CA, Mallory M, Achim CL, McCutchan JA, Nelson JA, Atkinson JH, Grant I.** 1997. Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. HNRC Group. The HIV Neurobehavioral Research Center. Ann Neurol **42:**963-972.

13. **McPhail ME, Robertson KR.** 2011. Neurocognitive impact of antiretroviral treatment: thinking long-term. Curr HIV/AIDS Rep **8:**249-256.
14. **Simioni S, Cavassini M, Annoni JM, Rimbault Abraham A, Bourquin I, Schiffer V, Calmy A, Chave JP, Giacobini E, Hirschel B, Du Pasquier RA.** 2010. Cognitive dysfunction in HIV patients despite long-standing suppression of viremia. AIDS **24:**1243-1250.
15. **Desplats P, Dumaop W, Smith D, Adame A, Everall I, Letendre S, Ellis R, Cherner M, Grant I, Masliah E.** 2013. Molecular and pathologic insights from latent HIV-1 infection in the human brain. Neurology **80:**1415-1423.
16. **Kraft-Terry SD, Buch SJ, Fox HS, Gendelman HE.** 2009. A coat of many colors: neuroimmune crosstalk in human immunodeficiency virus infection. Neuron **64:**133-145.
17. **Levine AJ, Soontornniyomkij V, Achim CL, Masliah E, Gelman BB, Sinsheimer JS, Singer EJ, Moore DJ.** 2016. Multilevel analysis of neuropathogenesis of neurocognitive impairment in HIV. J Neurovirol **22:**431-441.
18. **Gelman BB, Chen T, Lisinicchia JG, Soukup VM, Carmical JR, Starkey JM, Masliah E, Commins DL, Brandt D, Grant I, Singer EJ, Levine AJ, Miller J, Winkler JM, Fox HS, Luxon BA, Morgello S.** 2012. The National NeuroAIDS Tissue Consortium brain gene array: two types of HIV-associated neurocognitive impairment. PLoS One **7:**e46178.
19. **Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP.** 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A **102:**15545-15550.
20. **Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P.** 2015. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst **1:**417-425.
21. **Lim WK, Wang K, Lefebvre C, Califano A.** 2007. Comparative analysis of microarray normalization procedures: effects on reverse engineering gene networks. Bioinformatics **23:**i282-288.
22. **Schrier RD, Hong S, Crescini M, Ellis R, Perez-Santiago J, Spina C, Letendre S, Group H.** 2015. Cerebrospinal fluid (CSF) CD8+ T-cells that express interferon-gamma contribute to HIV associated neurocognitive disorders (HAND). PLoS One **10:**e0116526.
23. **Gelman BB, Lisinicchia JG, Morgello S, Masliah E, Commins D, Achim CL, Fox HS, Kolson DL, Grant I, Singer E, Yiannoutsos CT, Sherman S, Gensler G, Moore DJ, Chen T, Soukup VM.** 2013. Neurovirological correlation with HIV-associated neurocognitive disorders and encephalitis in a HAART-era cohort. J Acquir Immune Defic Syndr **62:**487-495.
24. **Gao HM, Hong JS.** 2008. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends Immunol **29:**357-365.
25. **Rho MB, Wesselingh S, Glass JD, McArthur JC, Choi S, Griffin J, Tyor WR.** 1995. A potential role for interferon-alpha in the pathogenesis of HIV-associated dementia. Brain Behav Immun **9:**366-377.
26. **Zheng J, Thylin MR, Persidsky Y, Williams CE, Cotter RL, Zink W, Ryan L, Ghorpade A, Lewis K, Gendelman HE.** 2001. HIV-1 infected immune

competent mononuclear phagocytes influence the pathways to neuronal demise. Neurotox Res **3:**461-484.

27. **Tyor WR, Glass JD, Griffin JW, Becker PS, McArthur JC, Bezman L, Griffin DE.** 1992. Cytokine expression in the brain during the acquired immunodeficiency syndrome. Ann Neurol **31:**349-360.
28. **Perrella O, Carreiri PB, Perrella A, Sbreglia C, Gorga F, Guarnaccia D, Tarantino G.** 2001. Transforming growth factor beta-1 and interferon-alpha in the AIDS dementia complex (ADC): possible relationship with cerebral viral load? Eur Cytokine Netw **12:**51-55.
29. **Ryan LA, Cotter RL, Zink WE, 2nd, Gendelman HE, Zheng J.** 2002. Macrophages, chemokines and neuronal injury in HIV-1-associated dementia. Cell Mol Biol (Noisy-le-grand) **48:**137-150.
30. **Krivine A, Force G, Servan J, Cabee A, Rozenberg F, Dighiero L, Marguet F, Lebon P.** 1999. Measuring HIV-1 RNA and interferon-alpha in the cerebrospinal fluid of AIDS patients: insights into the pathogenesis of AIDS Dementia Complex. J Neurovirol **5:**500-506.
31. **Boehm U, Klamp T, Groot M, Howard JC.** 1997. Cellular responses to interferon-gamma. Annu Rev Immunol **15:**749-795.
32. **Burrone OR, Milstein C.** 1982. Control of HLA-A,B,C synthesis and expression in interferon-treated cells. EMBO J **1:**345-349.
33. **Calabresi PA, Tranquill LR, Dambrosia JM, Stone LA, Maloni H, Bash CN, Frank JA, McFarland HF.** 1997. Increases in soluble VCAM-1 correlate with a decrease in MRI lesions in multiple sclerosis treated with interferon beta-1b. Ann Neurol **41:**669-674.
34. **Hofer MJ, Campbell IL.** 2013. Type I interferon in neurological disease-the devil from within. Cytokine Growth Factor Rev **24:**257-267.
35. **Campbell IL, Krucker T, Steffensen S, Akwa Y, Powell HC, Lane T, Carr DJ, Gold LH, Henriksen SJ, Siggins GR.** 1999. Structural and functional neuropathology in transgenic mice with CNS expression of IFN-alpha. Brain Res **835:**46-61.
36. **Blank T, Detje CN, Spiess A, Hagemeyer N, Brendecke SM, Wolfart J, Staszewski O, Zoller T, Papageorgiou I, Schneider J, Paricio-Montesinos R, Eisel UL, Manahan-Vaughan D, Jansen S, Lienenklaus S, Lu B, Imai Y, Muller M, Goelz SE, Baker DP, Schwaninger M, Kann O, Heikenwalder M, Kalinke U, Prinz M.** 2016. Brain Endothelial- and Epithelial-Specific Interferon Receptor Chain 1 Drives Virus-Induced Sickness Behavior and Cognitive Impairment. Immunity **44:**901-912.
37. **Filiano AJ, Xu Y, Tustison NJ, Marsh RL, Baker W, Smirnov I, Overall CC, Gadani SP, Turner SD, Weng Z, Peerzade SN, Chen H, Lee KS, Scott MM, Beenhakker MP, Litvak V, Kipnis J.** 2016. Unexpected role of interferon-gamma in regulating neuronal connectivity and social behaviour. Nature doi:10.1038/nature18626.
38. **Schippling S, O'Connor P, Knappertz V, Pohl C, Bogumil T, Suarez G, Cook S, Filippi M, Hartung HP, Comi G, Jeffery DR, Kappos L, Goodin DS, Arnason B.** 2016. Incidence and course of depression in multiple sclerosis in the multinational BEYOND trial. J Neurol **263:**1418-1426.

39. **Shapshak P, Duncan R, Minagar A, Rodriguez de la Vega P, Stewart RV, Goodkin K.** 2004. Elevated expression of IFN-gamma in the HIV-1 infected brain. Front Biosci **9:**1073-1081.
40. **Webster NL, Crowe SM.** 2006. Matrix metalloproteinases, their production by monocytes and macrophages and their potential role in HIV-related diseases. J Leukoc Biol **80:**1052-1066.
41. **Conant K, Lim ST, Randall B, Maguire-Zeiss KA.** 2012. Matrix metalloproteinase dependent cleavage of cell adhesion molecules in the pathogenesis of CNS dysfunction with HIV and methamphetamine. Curr HIV Res **10:**384-391.
42. **Dziembowska M, Wlodarczyk J.** 2012. MMP9: a novel function in synaptic plasticity. Int J Biochem Cell Biol **44:**709-713.
43. **Nottet HS.** 1999. Interactions between macrophages and brain microvascular endothelial cells: role in pathogenesis of HIV-1 infection and blood - brain barrier function. J Neurovirol **5:**659-669.
44. **Louboutin JP, Strayer DS.** 2012. Blood-brain barrier abnormalities caused by HIV-1 gp120: mechanistic and therapeutic implications. ScientificWorldJournal **2012:**482575.
45. **Stuart MJ, Baune BT.** 2014. Chemokines and chemokine receptors in mood disorders, schizophrenia, and cognitive impairment: a systematic review of biomarker studies. Neurosci Biobehav Rev **42:**93-115.
46. **McManus C, Berman JW, Brett FM, Staunton H, Farrell M, Brosnan CF.** 1998. MCP-1, MCP-2 and MCP-3 expression in multiple sclerosis lesions: an immunohistochemical and in situ hybridization study. J Neuroimmunol **86:**20-29.
47. **Lalli MA, Bettcher BM, Arcila ML, Garcia G, Guzman C, Madrigal L, Ramirez L, Acosta-Uribe J, Baena A, Wojta KJ, Coppola G, Fitch R, de Both MD, Huentelman MJ, Reiman EM, Brunkow ME, Glusman G, Roach JC, Kao AW, Lopera F, Kosik KS.** 2015. Whole-genome sequencing suggests a chemokine gene cluster that modifies age at onset in familial Alzheimer's disease. Mol Psychiatry **20:**1294-1300.
48. **Thames AD, Briones MS, Magpantay LI, Martinez-Maza O, Singer EJ, Hinkin CH, Morgello S, Gelman BB, Moore DJ, Heizerling K, Levine AJ.** 2015. The role of chemokine C-C motif ligand 2 genotype and cerebrospinal fluid chemokine C-C motif ligand 2 in neurocognition among HIV-infected patients. AIDS **29:**1483-1491.
49. **Najera JA, Bustamante EA, Bortell N, Morsey B, Fox HS, Ravasi T, Marcondes MC.** 2016. Methamphetamine abuse affects gene expression in brain-derived microglia of SIV-infected macaques to enhance inflammation and promote virus targets. BMC Immunol **17:**7.
50. **Gersten M, Alirezaei M, Marcondes MC, Flynn C, Ravasi T, Ideker T, Fox HS.** 2009. An integrated systems analysis implicates EGR1 downregulation in simian immunodeficiency virus encephalitis-induced neural dysfunction. J Neurosci **29:**12467-12476.
51. **Marciniak E, Faivre E, Dutar P, Alves Pires C, Demeyer D, Caillierez R, Laloux C, Buee L, Blum D, Humez S.** 2015. The Chemokine MIP-1alpha/CCL3 impairs mouse hippocampal synaptic transmission, plasticity and memory. Sci Rep **5:**15862.

583 52. **Brook M, Tomlinson GH, Miles K, Smith RW, Rossi AG, Hiemstra PS, van 't**
584 **Wout EF, Dean JL, Gray NK, Lu W, Gray M.** 2016. Neutrophil-derived alpha
585 defensins control inflammation by inhibiting macrophage mRNA translation. Proc
586 Natl Acad Sci U S A **113:**4350-4355.
587 53. **Williams WM, Torres S, Siedlak SL, Castellani RJ, Perry G, Smith MA, Zhu**
588 **X.** 2013. Antimicrobial peptide beta-defensin-1 expression is upregulated in
589 Alzheimer's brain. J Neuroinflammation **10:**127.
590 54. **Zyskind JW, Wang Y, Cho G, Ting JH, Kolson DL, Lynch DR, Jordan-Sciutto**
591 **KL.** 2015. E2F1 in neurons is cleaved by calpain in an NMDA receptor-
592 dependent manner in a model of HIV-induced neurotoxicity. J Neurochem
593 **132:**742-755.
594 55. **Ande A, Sinha N, Rao PS, McArthur CP, Ayuk L, Achu PN, Njinda A, Kumar**
595 **A, Kumar S.** 2015. Enhanced oxidative stress by alcohol use in HIV+ patients:
596 possible involvement of cytochrome P450 2E1 and antioxidant enzymes. AIDS
597 Res Ther **12:**29.
598 56. **Aylward EH, Brettschneider PD, McArthur JC, Harris GJ, Schlaepfer TE,**
599 **Henderer JD, Barta PE, Tien AY, Pearlson GD.** 1995. Magnetic resonance
600 imaging measurement of gray matter volume reductions in HIV dementia. Am J
601 Psychiatry **152:**987-994.
602 57. **Gongvatana A, Schweinsburg BC, Taylor MJ, Theilmann RJ, Letendre SL,**
603 **Alhassoon OM, Jacobus J, Woods SP, Jernigan TL, Ellis RJ, Frank LR,**
604 **Grant I, Charter G.** 2009. White matter tract injury and cognitive impairment in
605 human immunodeficiency virus-infected individuals. J Neurovirol **15:**187-195.
606 58. **Stubbe-Drger B, Deppe M, Mohammadi S, Keller SS, Kugel H, Gregor N,**
607 **Evers S, Young P, Ringelstein EB, Arendt G, Knecht S, Husstedt IW,**
608 **German Competence Network HA.** 2012. Early microstructural white matter
609 changes in patients with HIV: a diffusion tensor imaging study. BMC Neurol
610 **12:**23.
611 59. **Everall IP, Heaton RK, Marcotte TD, Ellis RJ, McCutchan JA, Atkinson JH,**
612 **Grant I, Mallory M, Masliah E.** 1999. Cortical synaptic density is reduced in mild
613 to moderate human immunodeficiency virus neurocognitive disorder. HNRC
614 Group. HIV Neurobehavioral Research Center. Brain Pathol **9:**209-217.
615 60. **Ipser JC, Brown GG, Bischoff-Grethe A, Connolly CG, Ellis RJ, Heaton RK,**
616 **Grant I, Translational Methamphetamine ARCG.** 2015. HIV infection is
617 associated with attenuated frontostriatal intrinsic connectivity: a preliminary
618 study. J Int Neuropsychol Soc **21:**203-213.
619 61. **Steinbrink F, Evers S, Buerke B, Young P, Arendt G, Koutsilieri E, Reichelt**
620 **D, Lohmann H, Husstedt IW, German Competence Network HA.** 2013.
621 Cognitive impairment in HIV infection is associated with MRI and CSF pattern of
622 neurodegeneration. Eur J Neurol **20:**420-428.
623 62. **Kuper M, Rabe K, Esser S, Gizewski ER, Husstedt IW, Maschke M,**
624 **Obermann M.** 2011. Structural gray and white matter changes in patients with
625 HIV. J Neurol **258:**1066-1075.
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627

628 **Captions to the Figures**

629 **Figure 1: A) Number of Pathways differentially regulated in each transition in**
630 **each brain region. ***Numbers in brackets indicate the number of pathways selectively
631 differentially regulated in that region for a particular transition as compared to the other
632 2 brain regions**. B) Bar plot showing the number of pathways significantly**
633 **differentially regulated per comparison (FDR < 0.01).** WM=White Matter; FC =
634 Frontal Cortex; BG = Basal Ganglia. Common pathways define pathways significant in
635 at least 2 regions.

636

637 **Figure 2: Differential regulation of IFN-related pathways in the groups of the**
638 **NNTC gene expression dataset. A) Gene expression evidence of interferon (IFN)**
639 **activation HIV-infected patients without NCI.** The diagrams show GSEA plots for 3
640 pathways representative of IFN activation in HIV-infected patients without NCI (group B,
641 left-hand side in the GSEA plot) as compared to uninfected controls (group A right-hand
642 side). Each pathway was tested in each region independently. WM = White Matter; FC
643 = Frontal Cortex; BG = Basal Ganglia. These pathways are indicative of type I IFN
644 activation and include IFN-related genes in **Top)** INTERFERON ALPHA BETA
645 SIGNALING, **Middle)** type II IFN activation (INTERFERON GAMMA SIGNALING), and
646 **Bottom)** ANTIGEN PRESENTATION FOLDING ASSEMBLY AND PEPTIDE LOADING
647 OF MHC CLASS I, a pathway involving several IFN-regulated MHC class I genes.
648 Significant changes in the expression of the pathways is indicated by the asymmetric
649 distribution of the genes in the geneset (vertical bars) and of the running enrichment
650 score plot (ES) (19). Genes participating in the enrichment (on the left of the leading
651 edge corresponding to the peak of the running enrichment in GSEA) are shown in Fig.

652 3. **B) Differential regulation of IFN-related pathways in the 4 groups of the NNTC**
653 **gene expression dataset.** Significant activation of pathways related to both type I and
654 type II IFN was seen in a brain region-specific pattern in HIV-infected patients without
655 NCI (group B) and in patients with HIVE (group D). **Top)** INTERFERON ALPHA BETA
656 SIGNALING in the white Matter, frontal cortex and basal ganglia; **Middle)**
657 INTERFERON GAMMA SIGNALING; and **Bottom)** ANTIGEN PRESENTATION
658 FOLDING ASSEMBLY AND PEPTIDE LOADING OF MHC CLASS I, in the same three
659 regions. Each plot represents the pathway activity (computed as an enrichment score)
660 in the 4 different phenotypes and is annotated with the FDR values of respective GSEA
661 comparisons.
662
663 **Figure 3: Genes differentially expressed in HIV-infected patients without NCI.** The
664 heatmap shows the genes in the leading edge of the pathways commonly dysregulated
665 in all 3 brain regions in HIV-infected patients without NCI as compared to uninfected
666 controls. We selected the 54 genes most differentially expressed (t-test, p-value < 0.01)
667 among the list of 128 genes belonging to the leading edges of the significant pathways
668 identified by the GSEA analysis in HIV-infected patients without NCI in comparison with
669 uninfected controls.
670
671 **Figure 4: Evidence of neuronal injury in HIV infected patients with HIV without**
672 **NCI. A)** and **B)** Downregulation of genes related to neuronal transmission in patients
673 with HIV without NCI (group B, left-hand side in the GSEA plot) vs. uninfected controls
674 (Group A). **C)** Upregulation of apoptotic-related pathways in the frontal cortex and basal
675 ganglia of HIV-infected patients without NCI (group B).

**Figure 5: White matter changes in HIV-infected patients with NCI without HIVE.** GSEA plots representative of induction of cytokines, chemokines and β-defensins in the HIV-infected patients with NCI without HIVE (group C in the NNTC gene expression dataset) as compared to uninfected controls.

**Figure 6: Gene expression evidence of neurodegeneration in in Frontal Cortex of patients with HIVE. A)** Huntington's disease-related pathways is downregulated in frontal cortex of patients with HIVE (group D) as compared to patients with NCI and no NIVE as well as with patients without NCI. Similarly, **(B)** and **(C)** show Parkinson's disease and Neuronal System pathways respectively. Each row represents the pathway activity (computed as an enrichment score) in the 4 different phenotypes and is annotated with the FDR values of respective GSEA comparisons, followed by GSEA plots of the pathways in the group D vs group B and group D vs. group C comparisons in frontal cortex.

691 **Table 1 Pathways differentially regulated in multiple brain regions in patients**
692 **infected with HIV without NCI as compared to uninfected controls.**

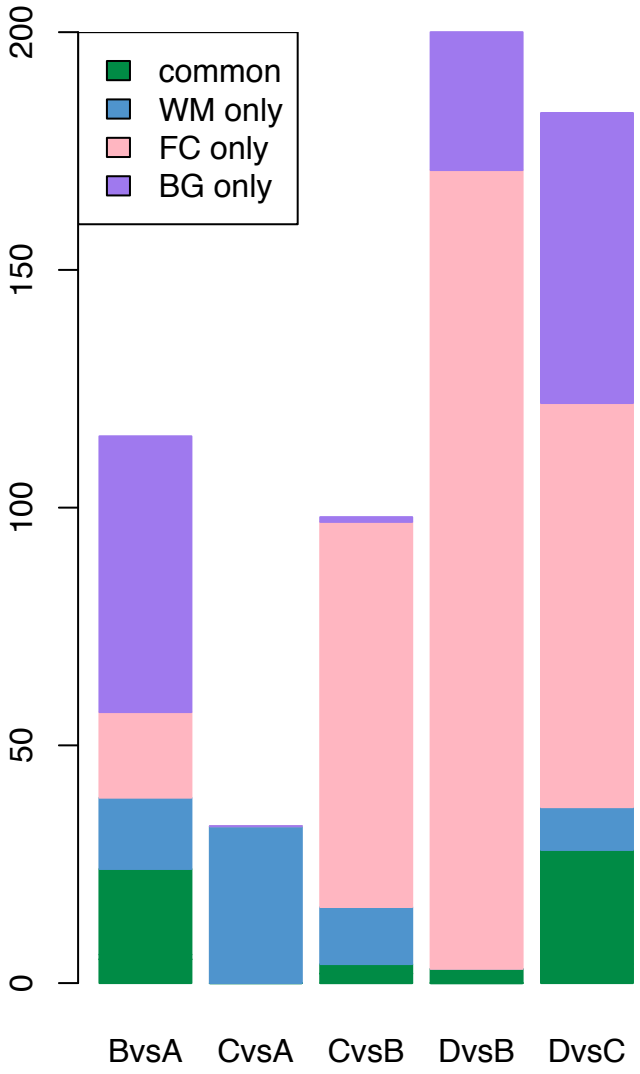
| Table 1 | WM | | FC | | BG | |
|---|---|---|---|---|---|---|
| **Pathways** | NES | FDR | NES | FDR | NES | FDR |
| REACTOME INTERFERON ALPHA BETA SIGNALING | 2.59 | 0 | 2.28 | 0 | 2.06 | 2E-3 |
| REACTOME INTERFERON SIGNALING | 2.53 | 0 | 2.20 | 0 | 2.10 | 7E-4 |
| REACTOME ANTIGEN PRESENTATION FOLDING ASSEMBLY AND PEPTIDE LOADING OF CLASS I MHC | 2.23 | 2E-4 | 2.20 | 0 | 2.19 | 0 |
| REACTOME ANTIGEN PROCESSING CROSS PRESENTATION | 1.97 | 8E-3 | 2.11 | 8E-4 | 2.46 | 0 |
| REACTOME CYTOKINE SIGNALING IN IMMUNE SYSTEM | 2.11 | 7E-4 | 1.95 | 7E-3 | 2.01 | 3E-3 |
| REACTOME INTERFERON GAMMA SIGNALING | 2.66 | 0 | 1.85 | 2E-2 | 1.93 | 6E-3 |
| REACTOME ER PHAGOSOME PATHWAY | 1.75 | 5E-2 | 2.21 | 0 | 2.45 | 0 |
| REACTOME ANTIVIRAL MECHANISM BY IFN STIMULATED GENES | 1.42 | 3E-01 | 2.10 | 1E-3 | 2.32 | 0 |
| REACTOME CLASS I MHC MEDIATED ANTIGEN PROCESSING PRESENTATION | 1.20 | 5E-1 | 2.05 | 2E-3 | 2.15 | 1E-4 |
| REACTOME HOST INTERACTIONS OF HIV FACTORS | 1.03 | 7E-1 | 2.15 | 0 | 2.19 | 0 |
| REACTOME ORC1 REMOVAL FROM CHROMATIN | 1.17 | 6E-1 | 1.94 | 8E-3 | 2.09 | 9E-4 |
| REACTOME APC C CDC20 MEDIATED DEGRADATION OF MITOTIC PROTEINS | 1.13 | 6E-1 | 1.93 | 8E-3 | 2.10 | 6E-4 |
| REACTOME REGULATION OF MRNA STABILITY BY PROTEINS THAT BIND AU RICH ELEMENTS | 1.05 | 7E-1 | 1.96 | 6E-3 | 2.04 | 2E-3 |
| REACTOME HIV INFECTION | -0.90 | 9E-1 | 2.17 | 0 | 2.11 | 6E-4 |
| REACTOME METABOLISM OF RNA | -0.96 | 8E-1 | 2.07 | 1E-3 | 2.03 | 2E-3 |
| KEGG NEUROACTIVE LIGAND RECEPTOR INTERACTION | -1.39 | 4E-1 | -2.34 | 2E-4 | -2.39 | 0 |
| REACTOME OLFACTORY SIGNALING PATHWAY | -0.85 | 9E-1 | -2.61 | 0 | -2.39 | 0 |
| REACTOME GPCR LIGAND BINDING | -1.13 | 6E-1 | -2.19 | 8E-04 | -2.37 | 0 |
| KEGG OLFACTORY TRANSDUCTION | -0.84 | 9E-1 | -2.46 | 0 | -2.37 | 0 |
| REACTOME CLASS A1 RHODOPSIN LIKE RECEPTORS | -1.04 | 7E-1 | -2.20 | 6E-4 | -2.38 | 0 |
| REACTOME LIGAND GATED ION CHANNEL TRANSPORT | -1.35 | 4E-1 | -2.11 | 3E-3 | -1.91 | 6E-3 |
| REACTOME PEPTIDE LIGAND BINDING RECEPTORS | -1.06 | 7E-1 | -2.02 | 7E-3 | -2.29 | 0 |
| NABA MATRISOME ASSOCIATED | 1.23 | 5E-1 | -2.05 | 5E-3 | -2.30 | 0 |
| NABA ECM REGULATORS | 1.01 | 8E-1 | -1.98 | 9E-3 | -1.89 | 8E-3 |

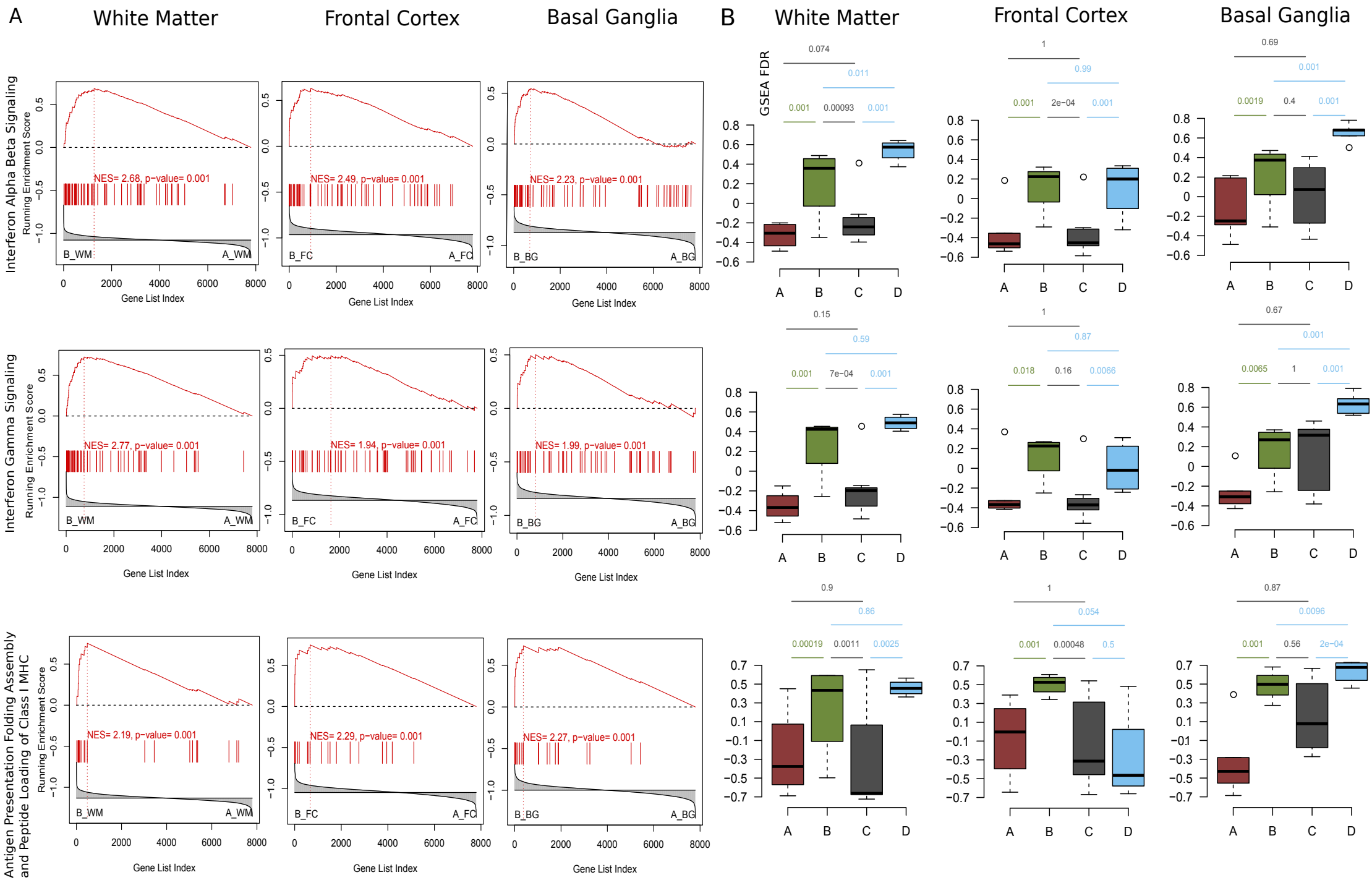
693 The table shows pathways dysregulated in at least 2 brain regions in patients infected
694 with HIV without NCI compared to uninfected controls. The dataset was interrogated for
695 pathway enrichment using the canonical pathways from the MSigDb C2 collection using
696 GSEA. The GSEA pathway analysis results show gene expression changes involving
697 immune activation and neuronal injury that precede the onset of clinical NCI. Results
698 show gene expression changes indicative of immune activation characterized by IFN
699 and cytokine expression as well as evidence of neuronal suffering preceding NCI.
700
701 NES: normalized enrichment score; FDR: false discovery rate; WM: white matter; FC:
702 frontal cortex; BG: basal ganglia.
703

A

| Comparisons | Brain regions | | | Common pathways |
|---|---|---|---|---|
| | White Matter | Prefrontal Cortex | Basal Ganglia | |
| B vs. A | 21 (6)* | 41 (4) | 82 (1) | 24 |
| C vs. A | 33 (33) | 0 | 0 | 0 |
| C vs. B | 14 (0) | 85 (47) | 3 (1) | 2 |
| D vs. B | 0 | 171 (121) | 32 (16) | 3 |
| D vs. C | 24 (0) | 102 (37) | 88 (11) | 27 |

B




A
White Matter
Frontal Cortex
Basal Ganglia
B
White Matter
Frontal Cortex
Basal Ganglia
Interferon Alpha Beta Signaling
Interferon Gamma Signaling
Antigen Presentation Folding Assembly and Peptide Loading of Class I MHC
Running Enrichment Score
Gene List Index
NES= 2.68, p-value= 0.001
NES= 2.49, p-value= 0.001
NES= 2.23, p-value= 0.001
NES= 2.77, p-value= 0.001
NES= 1.94, p-value= 0.001
NES= 1.99, p-value= 0.001
NES= 2.19, p-value= 0.001
NES= 2.29, p-value= 0.001
NES= 2.27, p-value= 0.001
B_WM
A_WM
B_FC
A_FC
B_BG
A_BG
GSEA FDR
0.074
0.011
0.001
0.00093
0.001
1
0.99
0.001
2e-04
0.001
0.69
0.001
0.0019
0.4
0.001
0.15
0.59
0.001
7e-04
0.001
1
0.87
0.018
0.16
0.0066
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0.0065
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0.9
0.86
0.00019
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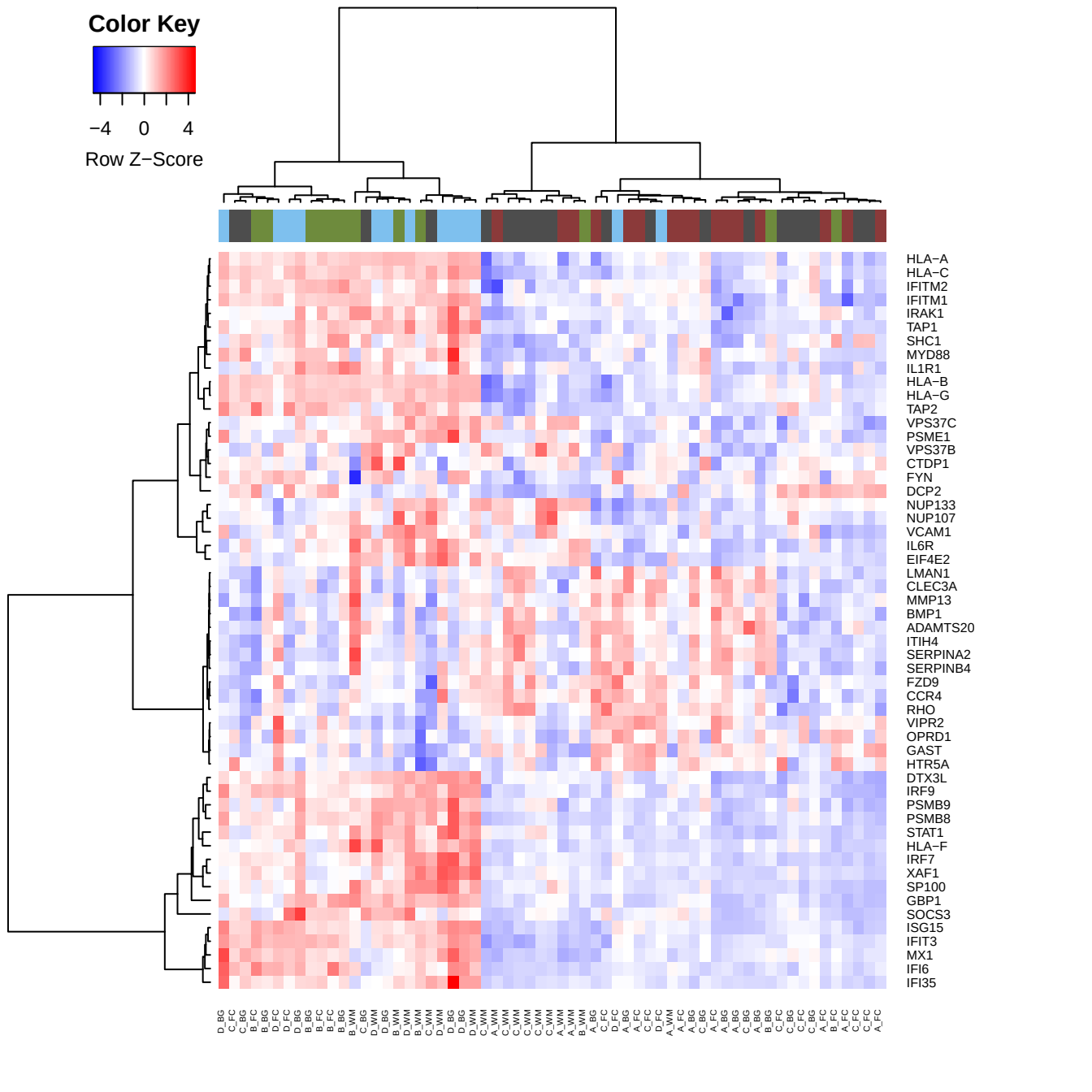
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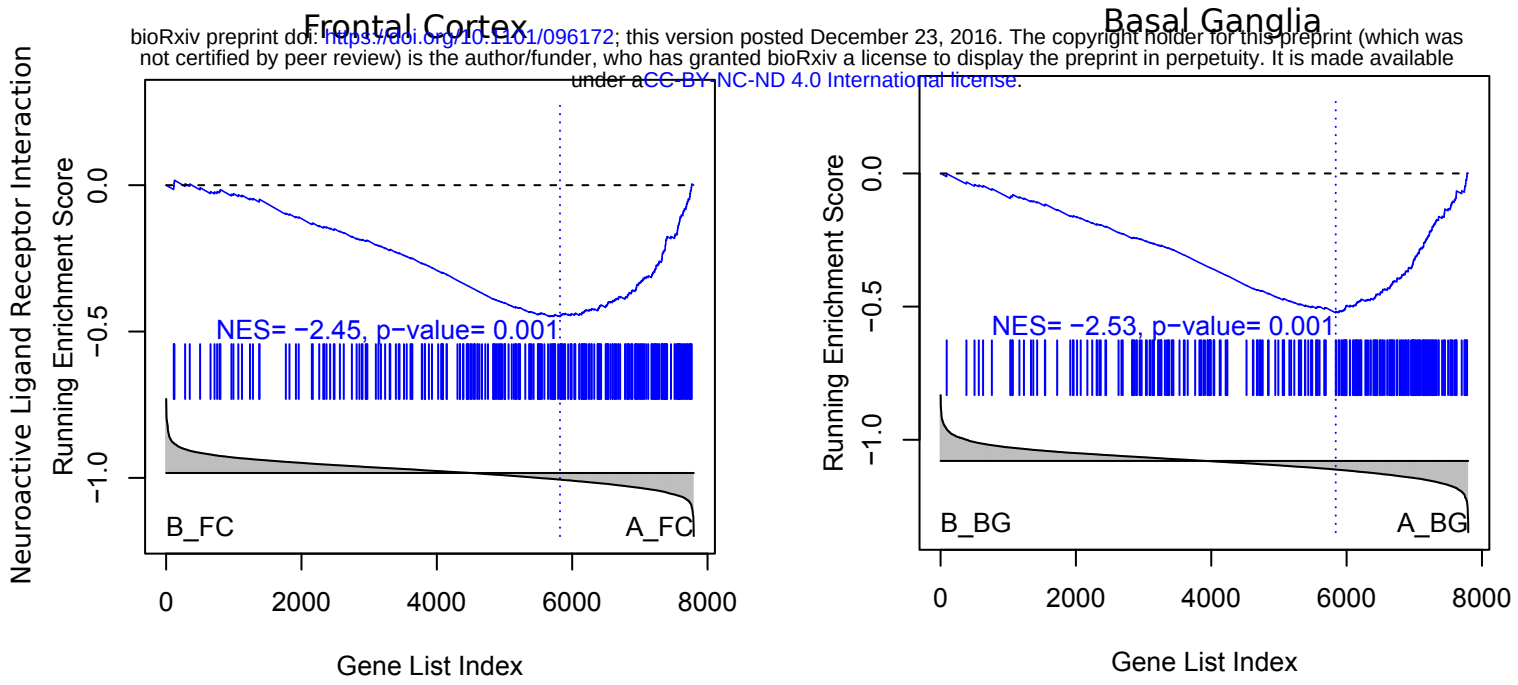
A

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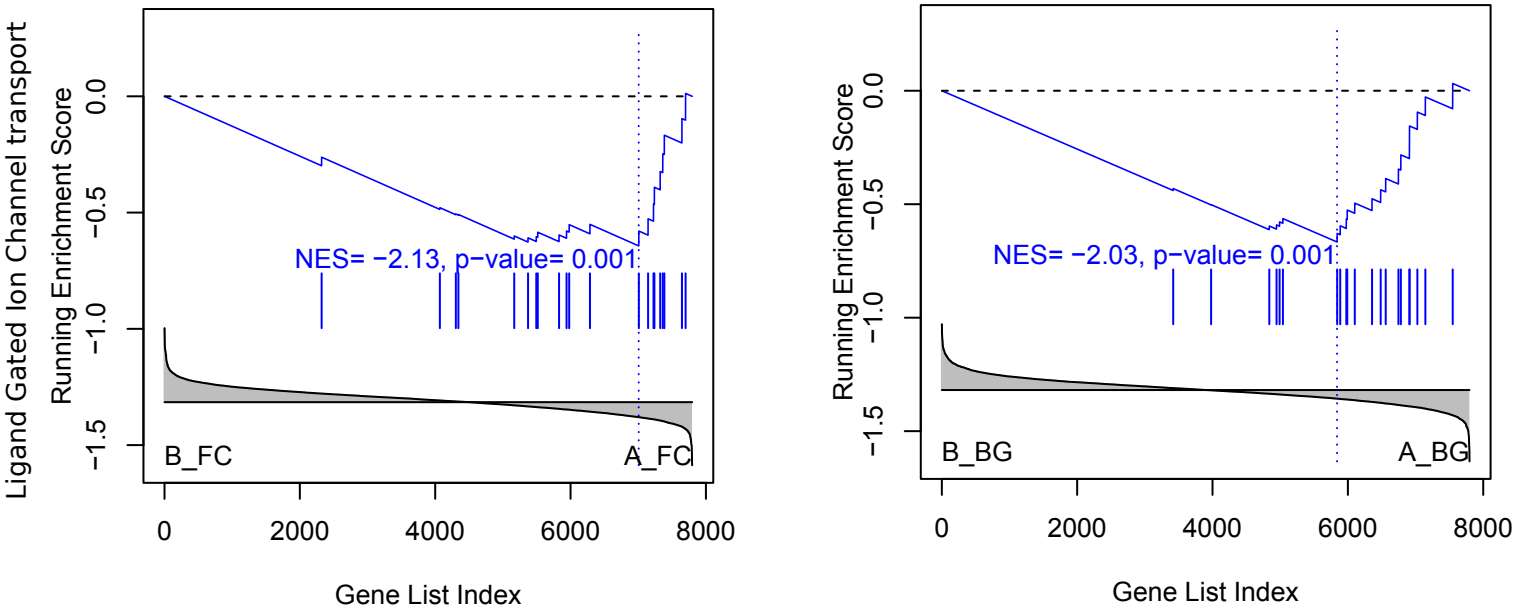


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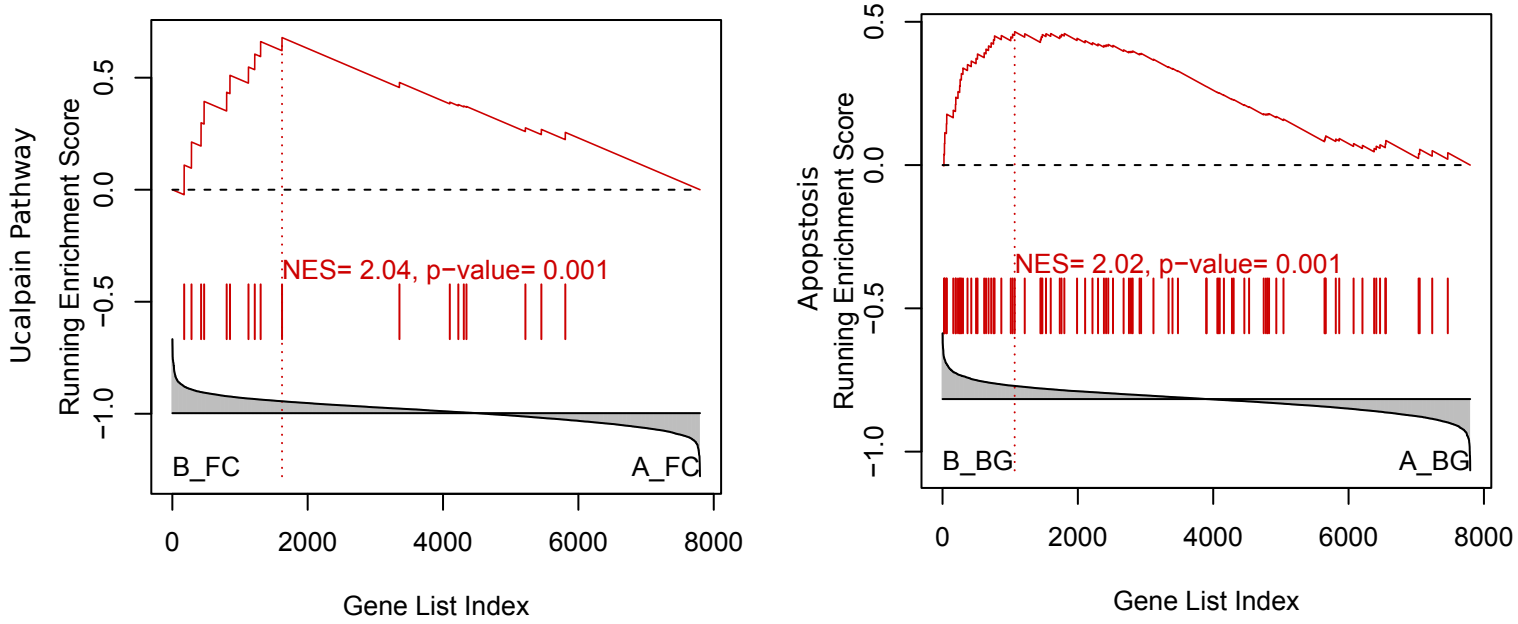

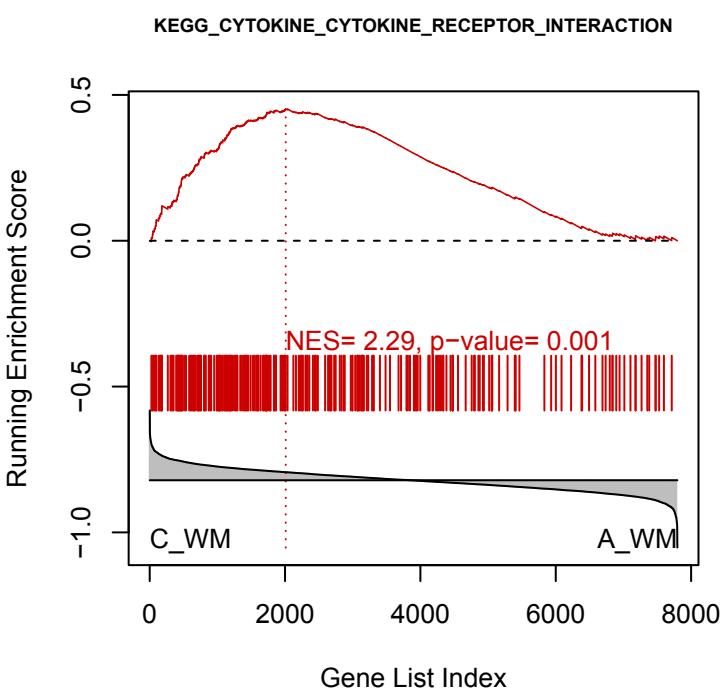


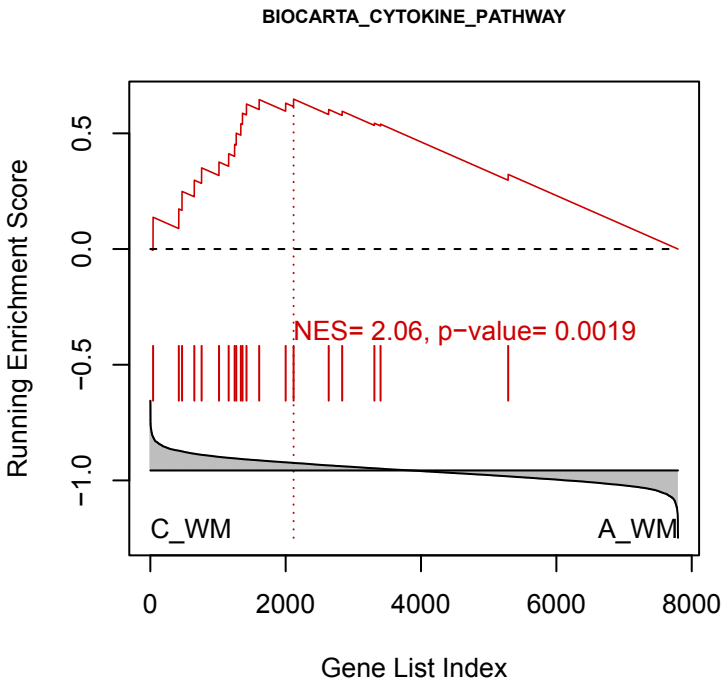


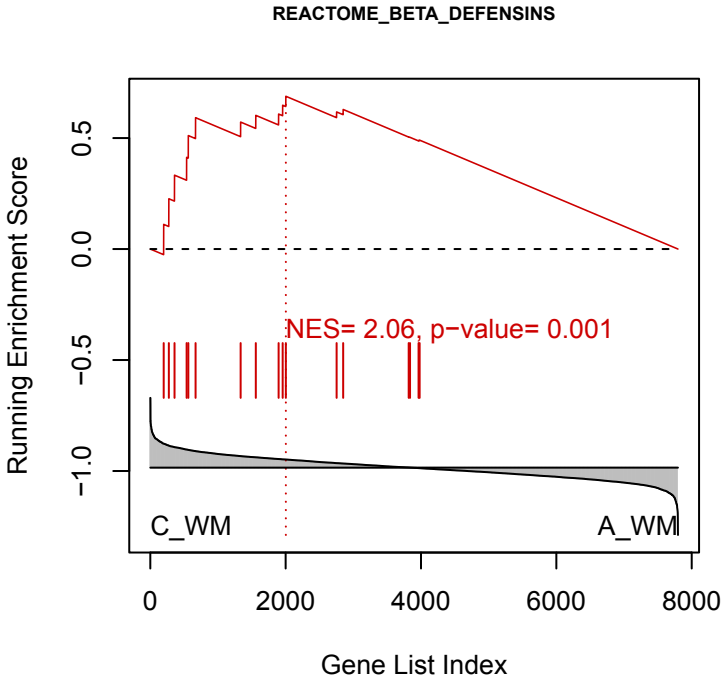


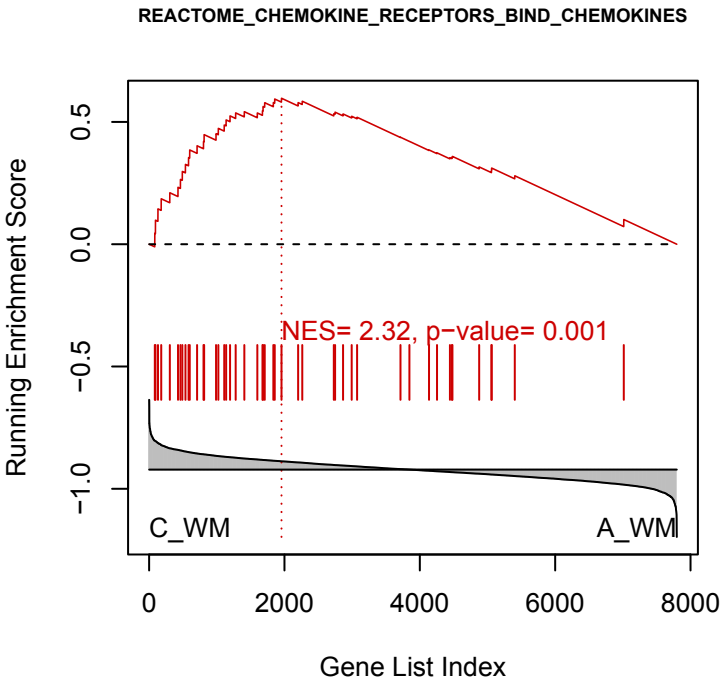

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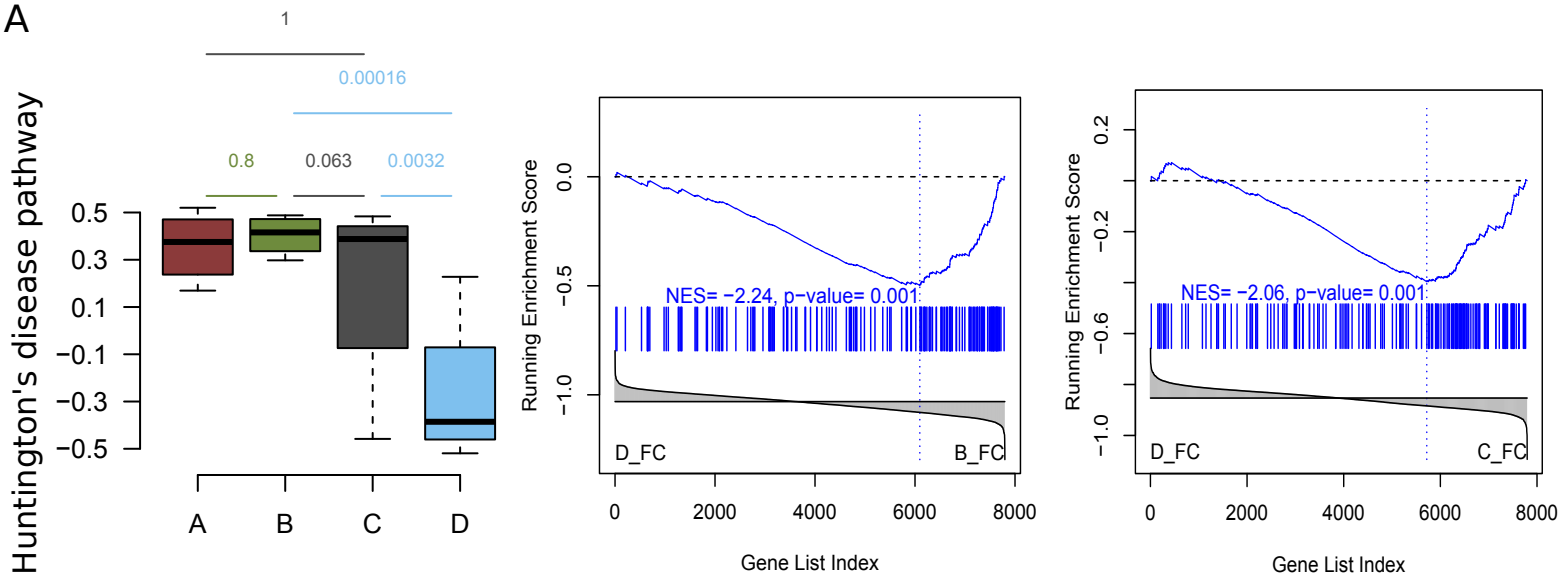
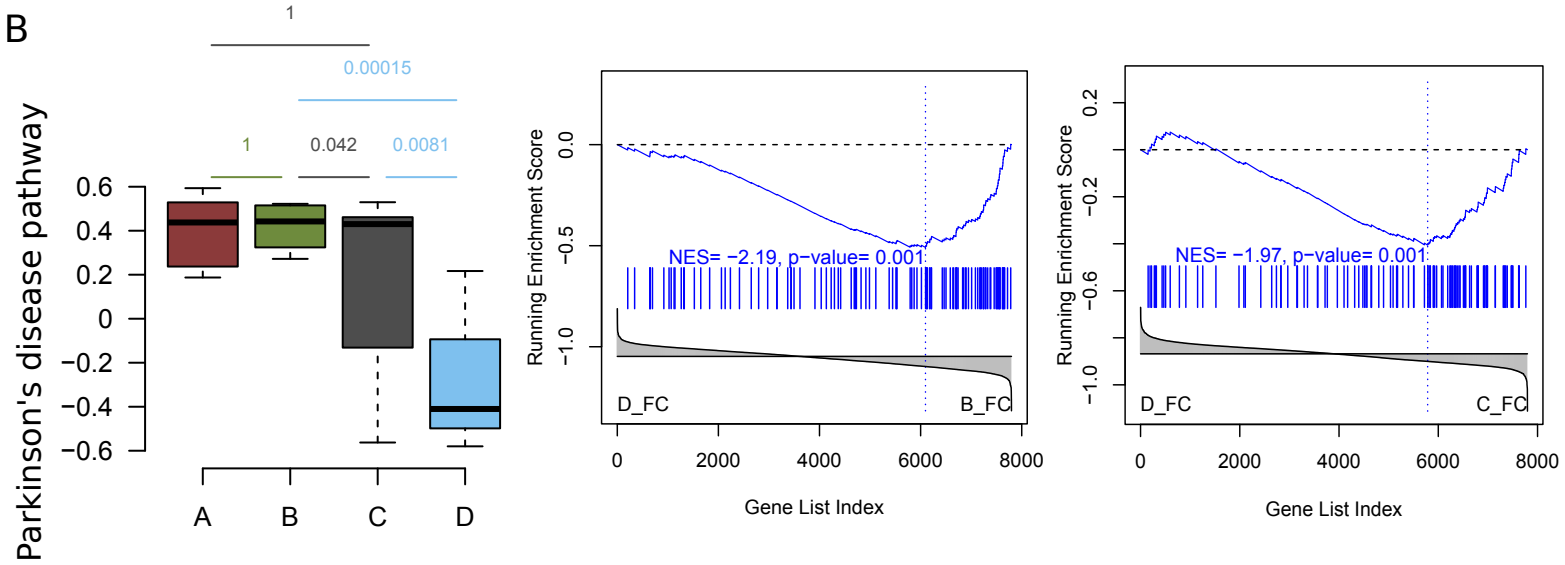



B




C