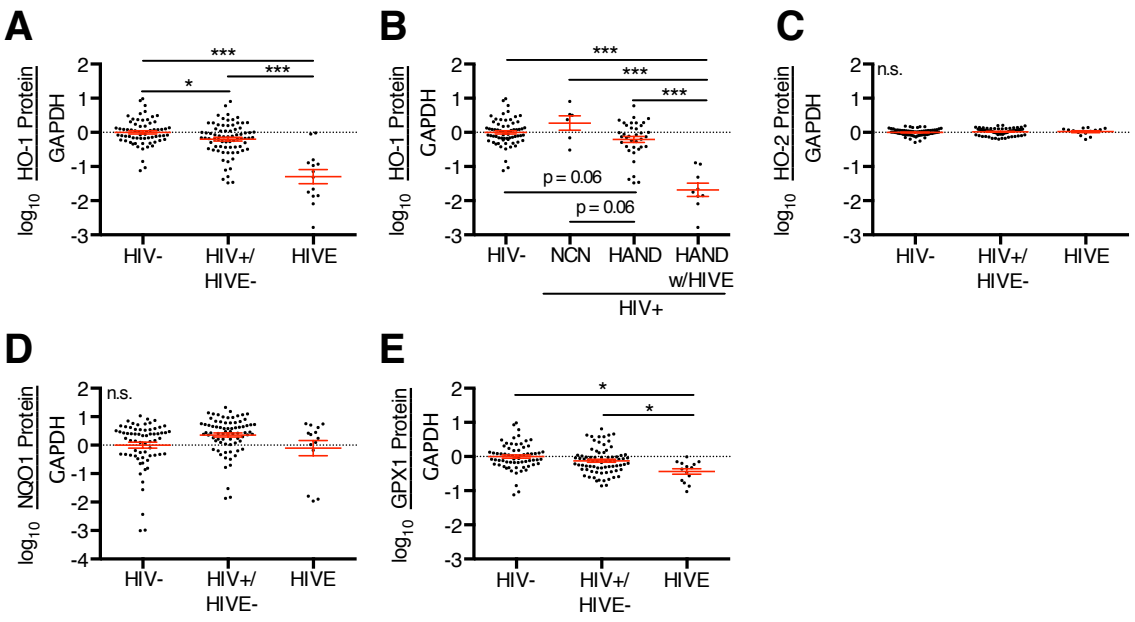
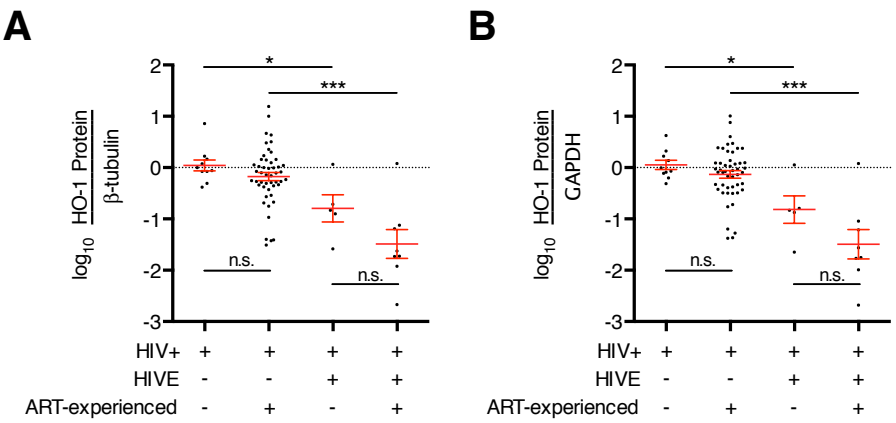
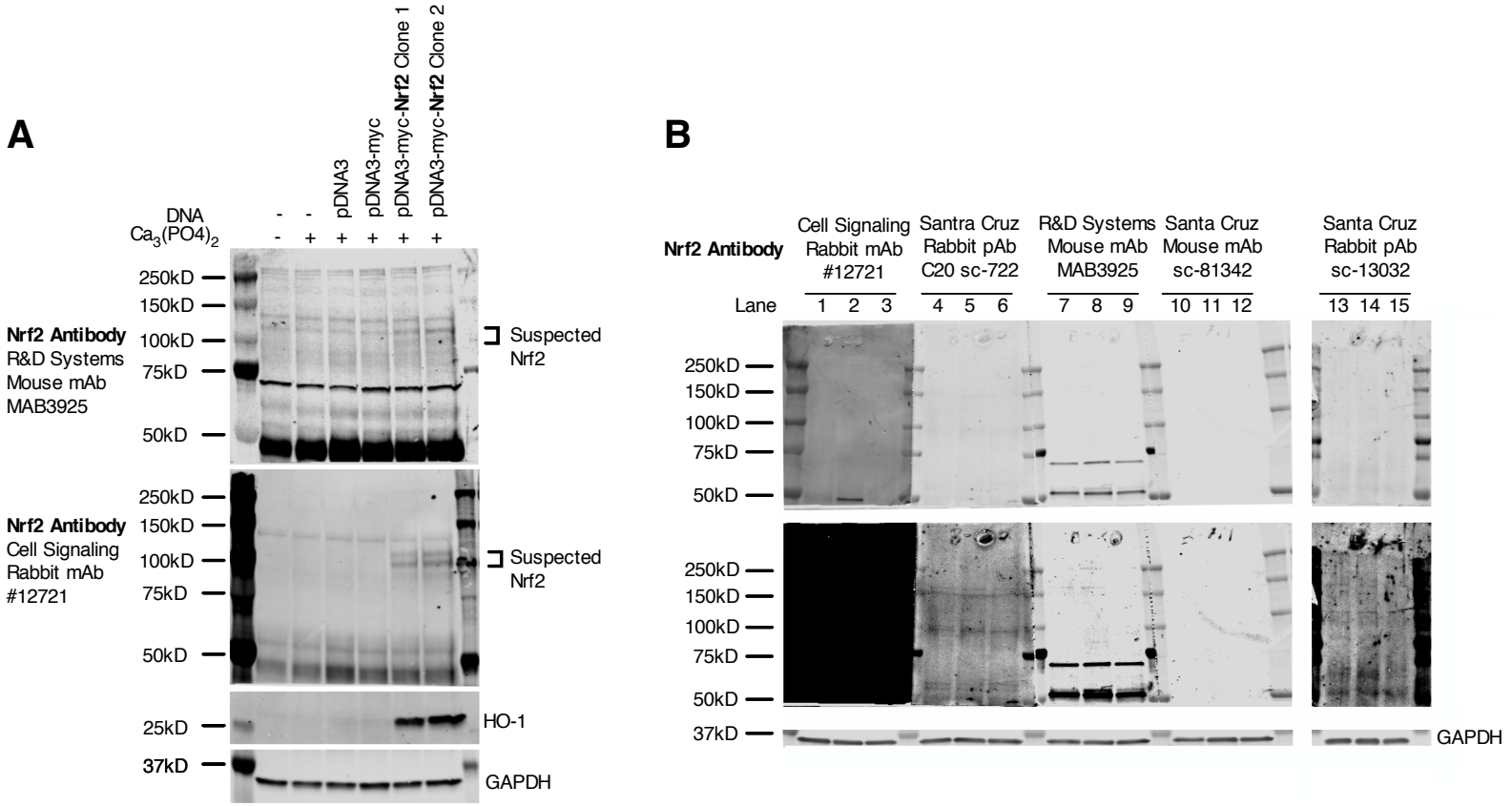


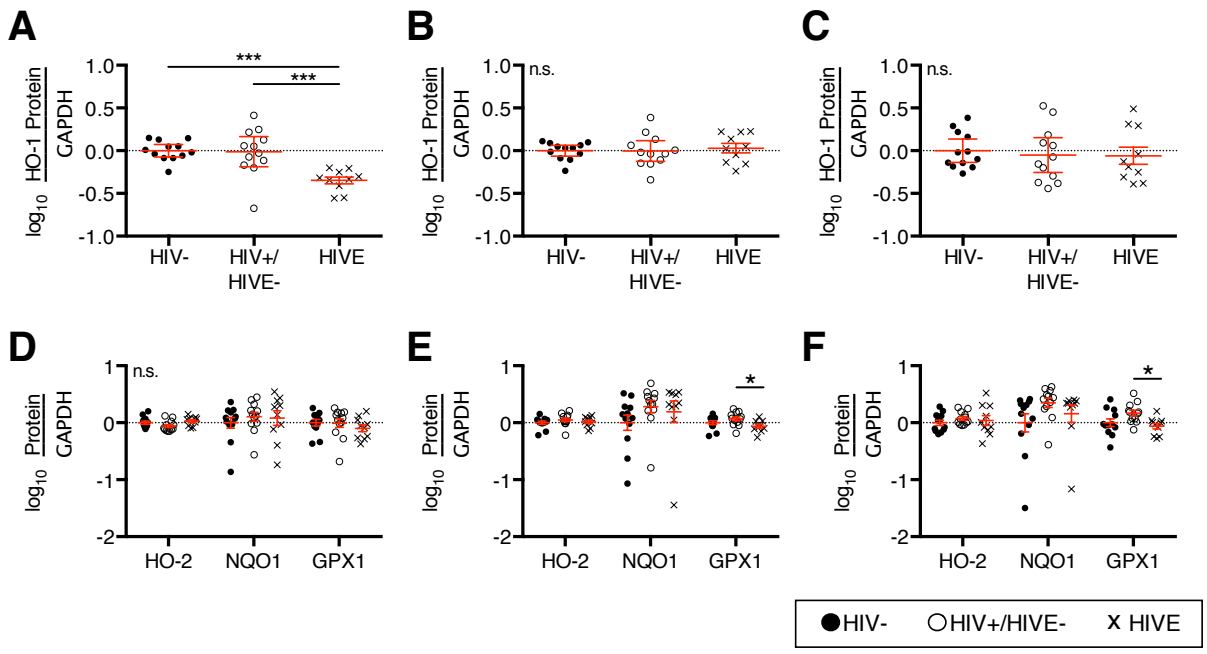
Supplemental Figure 1: HO-1 is deficient in the DLPFC of HIV-infected subjects. ARE protein expression was assessed by Western blot in 66 HIV-negative (HIV^-), 76 HIV-positive without HIVE ($HIV^+/HIVE^-$), and 14 HIV-positive with HIVE ($HIVE^+$) post-mortem DLPFC tissue samples. Of the 76 $HIV^+/HIVE^-$ subjects, 6 were confirmed to be neurocognitively normal (NCN) and 37 were diagnosed with HAND. (**A**) Representative Western blot of ARE proteins in the DLPFC of HIV^-, $HIV^+/HIVE^-$, and $HIVE^+$ subjects. Protein expression levels were quantified by densitometry analysis, normalized to GAPDH, and log transformed for comparison between groups for (**B-C**) HO-1, (**D**) NQO1, and (**E**) GPX1. The mean HIV- group protein expression was set to 0 (dotted line). Red lines indicate mean ± SEM. Groups were analyzed by ANOVA with post hoc Holm-Sidak test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



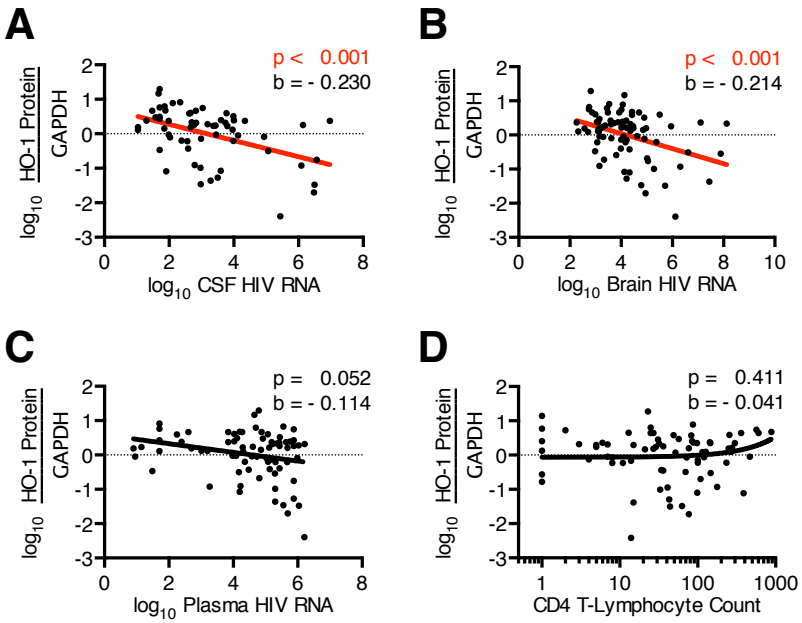
Supplemental Figure 2: ART exposure is not associated with DLPFC HO-1 deficiency in HIV$^+$ subjects with or without HIVE. HO-1 protein expression was assessed by Western blot and densitometry signal was normalized to (**A**) β-tubulin and (**B**) GAPDH in 10 ART-naive HIV$^+$ subjects without HIVE, 49 ART-experienced HIV$^+$ subjects without HIVE, 5 ART-naive subjects with HIVE, and 8 ART-experienced subjects with HIVE. No significant difference was observed in DLPFC HO-1 expression in ART-naive or ART experienced subjects in HIV$^+$ subjects with or without HIVE. DLPFC HO-1 expression was significantly reduced in HIVE subjects compared with HIV$^+$ subjects without HIVE in both the ART-naive and ART-experienced groups. The mean HIV- protein expression was set to 0 (dotted line). Red lines indicate mean ± SEM. Groups were analyzed by ANOVA with post hoc Holm-Sidak test. *$p < 0.05$, ***$p < 0.001$.



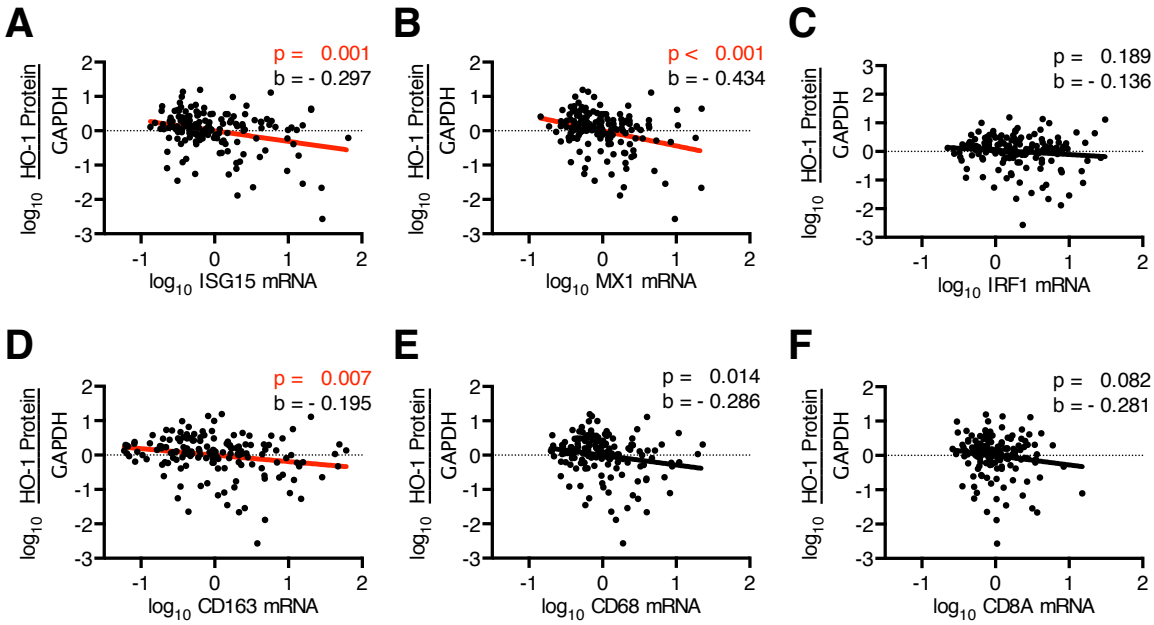
Supplemental Figure 3: Detection of Nrf2 protein by Western blot in Nrf2-overexpressing HEK cells and human DLPFC tissue. **A**) Empty pDNA3 vector, pDNA3-myc, or pDNA3-myc-Nrf2 were transfected into HEK cells by calcium phosphate transfection for 16 hours. Control HEK cells were left untreated or exposed to calcium phosphate transfection reagent without DNA. 48 hours after transfection cell lysates were collected and probed by Western blot for Nrf2 (mouse R&D systems 3925; rabbit Cell Signaling 12721), HO-1, and GAPDH. **B**) Prefrontal cortex protein lysates from 3 different HIV negative subjects were probed by Western blot with 5 commercially available antibodies directed against Nrf2 followed by probing with fluorescent secondary antibodies. Odyssey (LiCor) scanned Western blots show Nrf2 antibody immunoreactivity at a non-maximized (top blot) and maximized (middle blot) adjustment of the depiction of the fluorescence signal intensity using ImageStudio (LiCor). Bottom blots depict GAPDH immunoreactivity.



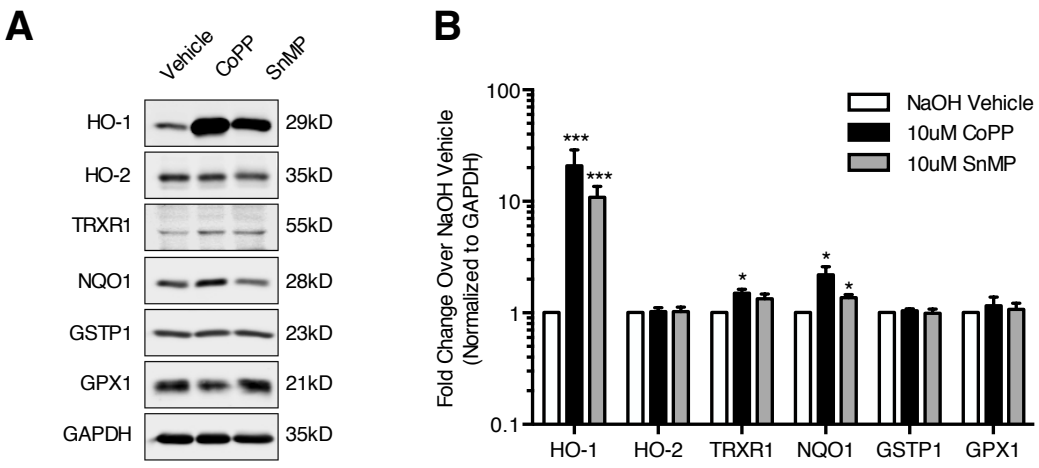
Supplemental Figure 4: HO-1 is deficient in the striatum of HIV-infected subjects with HIVE. HO-1 protein expression as determined by Western blot in the **A**) striatum (head of caudate), **B**) occipital cortex, and **C**) anterior cerebellum of 12 HIV^-, 12 $HIV^+/HIVE^-$, and 12 HIVE subjects. In the same cohort HO-2, NQO1, and GPX1 protein expression was determined by Western blot in the **D**) striatum, **E**) occipital cortex, and **F**) cerebellum. Protein expression levels were quantified by densitometry analysis, normalized to GAPDH, and log transformed for comparison between groups. The mean HIV- group protein expression was set to 0 (dotted line). Red lines indicate mean ± SEM. Groups were analyzed by ANOVA with post hoc Holm-Sidak test. *$p < 0.05$, ***$p < 0.001$



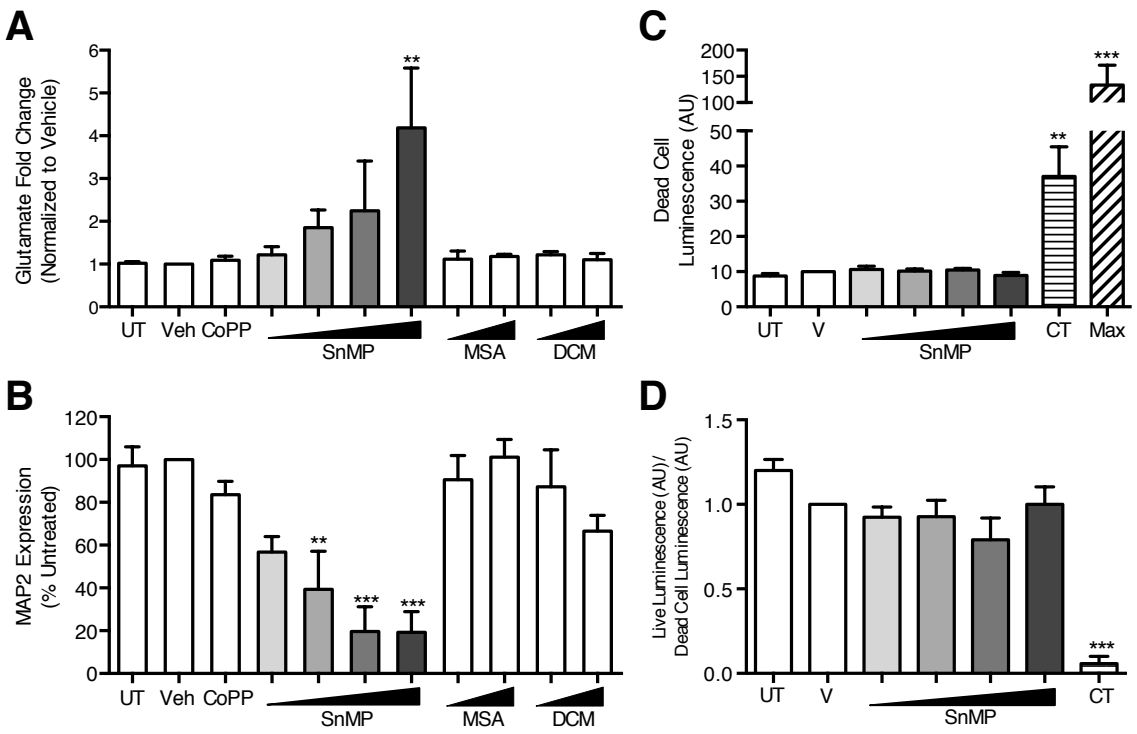
Supplemental Figure 5: DLPFC HO-1 protein expression correlates with CSF and brain HIV RNA levels. Samples were derived from the HIV$^+$ cohort for all subjects with detectable viral loads. Correlations were determined between DLPFC HO-1 protein expression in the DLPFC of HIV$^+$ subjects, as determined by Western blot and densitometry analysis normalized to GAPDH, and HIV RNA in (**A**) CSF, (**B**) brain parenchyma, and (**C**) plasma, and (**D**) plasma CD4 T-lymphocyte count in HIV$^+$ subjects. Associations were determined by multivariate linear regression with $\alpha = 0.01$. Red regression lines denote significant trends.



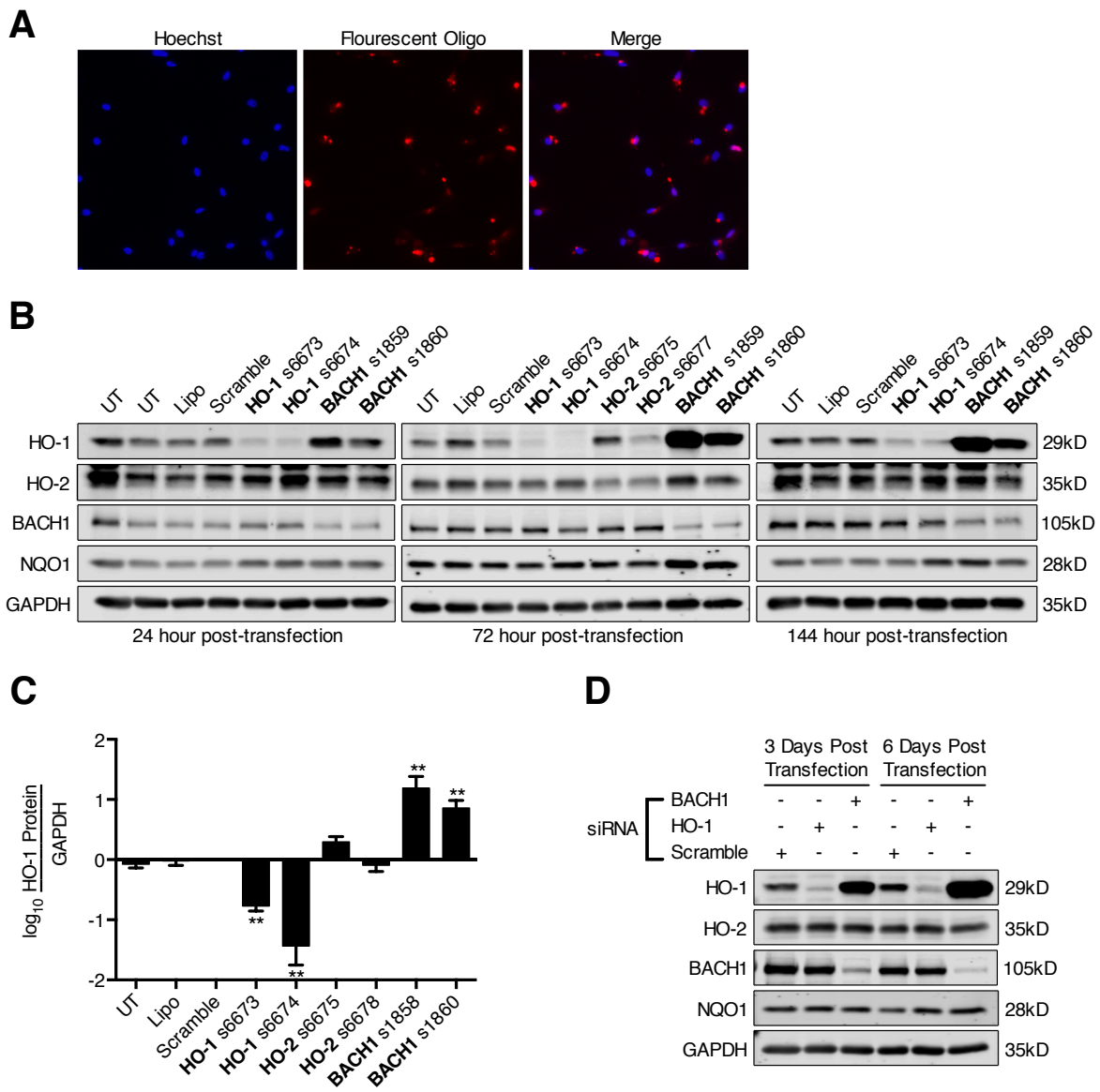
Supplemental Figure 6: DLPFC HO-1 protein expression correlates with brain innate immune responses and macrophage markers. Expression of mRNA was determined by RT-PCR with relative quantification to GAPDH. Correlations were determined between DLPFC HO-1 protein expression, as determined by Western blot and densitometry analysis normalized to GAPDH, and (**A**) *ISG15*, (**B**) *MX1*, (**C**) *IRF1*, (**D**) *CD163*, (**E**) *CD68*, and (**F**) *CD8A* mRNA. Associations were determined by multivariate linear regression with $\alpha = 0.01$. Red regression lines denote significant trends.



Supplemental Figure 7: CoPP and SnMP induce HO-1, but not HO-2, protein expression in MDM. Uninfected MDM were exposed to 10μM CoPP or SnMP for 24 hours and assessed for protein expression by Western blot. (**A**) Representative Western blot of HO-1, HO-2, TRXR1, NQO1, GSTP1, and GPX1 expression. (**B**) Quantification of Western blot expression by densitometry analysis. Data points represent mean ± SEM from 6 different MDM donors. Statistical comparisons were made by one-way ANOVA plus Hold-Sidak post hoc test. *$p < 0.05$, ***$p < 0.001$.

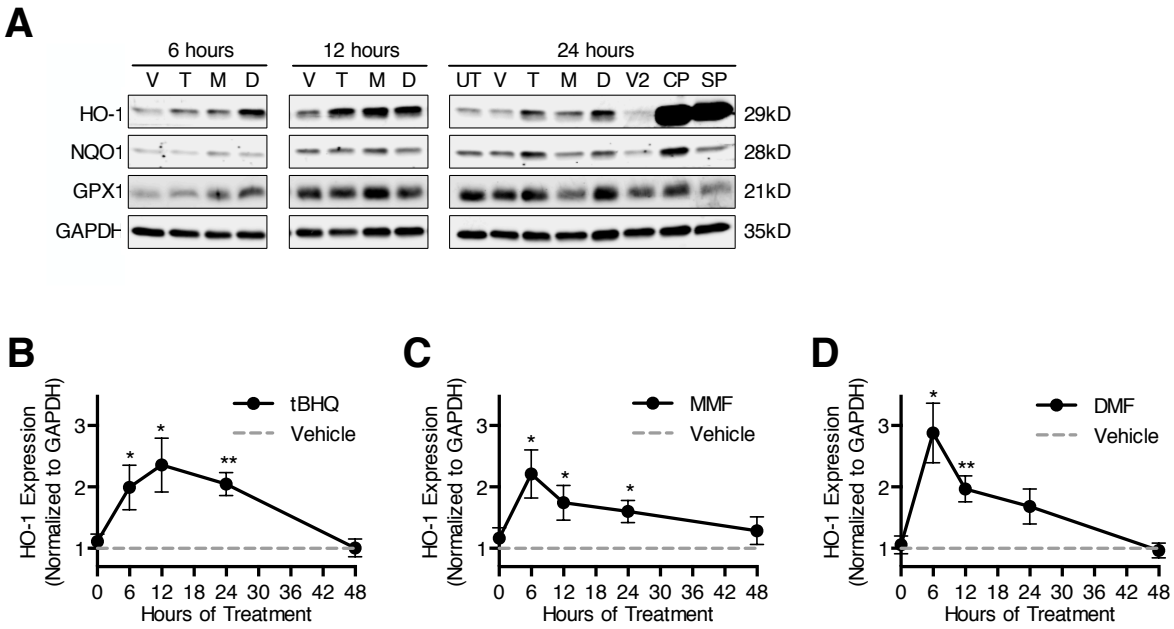


Supplemental Figure 8: Exposure of non-infected MDM to an inhibitor of HO-1 and HO-2 enzymatic activity (SnMP) enhances supernatant glutamate and neurotoxicity. Uninfected MDM from 4 different donors were exposed (6 days) to Vehicle (Veh), the HO-1 inhibitor SnMP (1, 5, 10, 20μM), the HO-1 expression inducer CoPP (10μM), the GPX1 inhibitor MSA (100 and 1000μM),the NQO1 inhibitor DCM (1 and 10μM), or left untreated (UT) and culture supernatants were assayed for (**A**) glutamate concentration and (**B**) neurotoxicity normalized to Vehicle. SnMP induced toxicity was quantified by (**C**) total dead cell luminescence and (**D**) the live/dead cell ratio in MDM cultures from 3 different donors. Camptothecin (CT; 6 hour exposure) and complete cell lysis (maximum cytotoxicity, Max) served as a positive controls. Values represent mean ± SEM. Statistical comparisons were made by one-way ANOVA plus Hold-Sidak post hoc test. $^{**}p < 0.01$, $^{***}p < 0.001$.

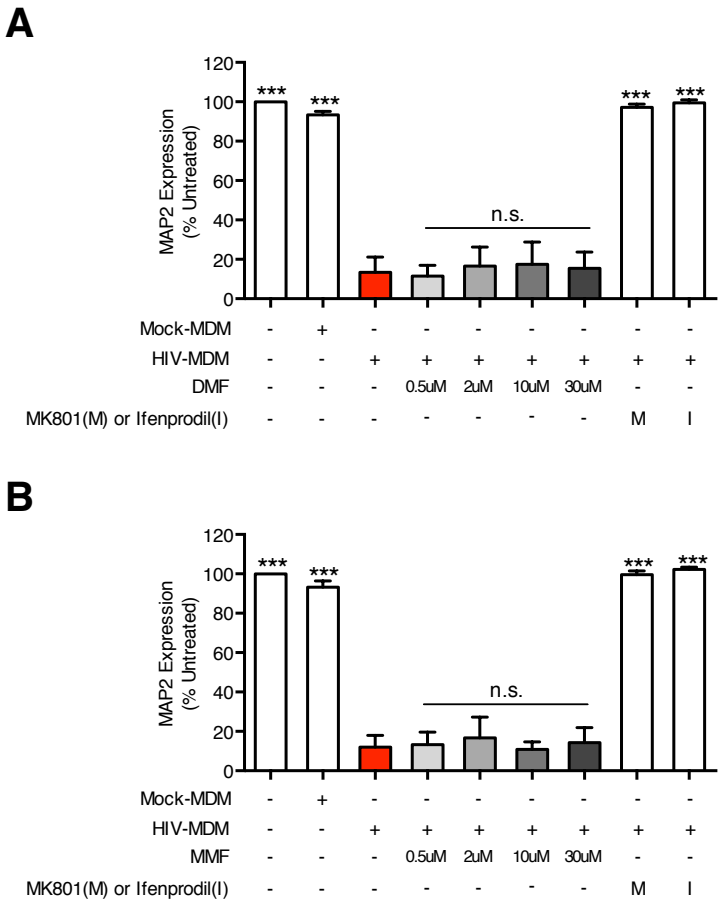


Supplemental Figure 9: HO-1 and BACH1 siRNAs effectively knockdown and induce HO-1 expression, respectively, in uninfected and infected MDM. 50nM of each siRNA was transfected into MDM using Lipofectamine RNAiMax. (**A**) Representative fluorescent microscopy images of MDM 24 hours post-transfection with fluorescent oligos (BLOCK-IT™ Alexa Fluor® Red Fluorescent Oligo). (**B**) Representative Western blot for HO-1, HO-2, BACH1, NQO1, and GAPDH at 24 hours, 72 hours, and 144 hours post-transfection with siRNA targeting either HO-1, HO-2, or BACH1. (**C**) Densitometry analysis of HO-1 Western blot protein expression 72 hours post siRNA transfection. Values represent log mean ± SEM of HO-1 expression normalized to GAPDH (Vehicle

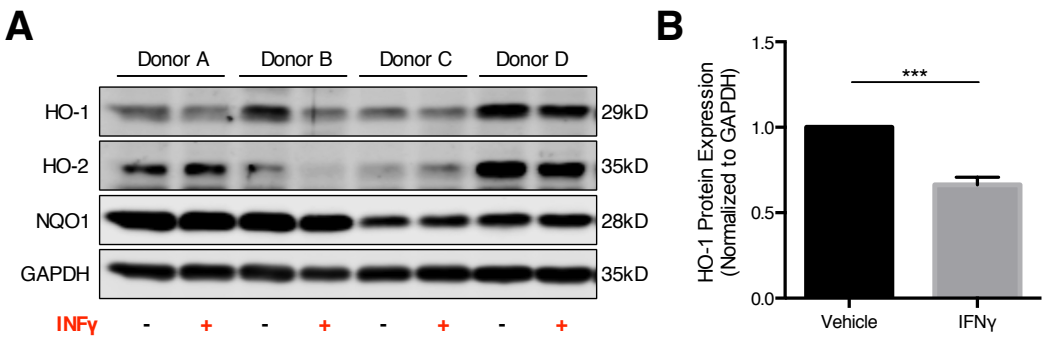
set to 0) from 4 different MDM donors. (**D**) Representative Western blot of HIV-infected MDM 3 days and 6 days post siRNA transfection. HIV-MDM were transfected with selected siRNA targeting HO-1 or BACH1 on day 6 post HIV infection. Protein lysates were either collected on day 9 post-infection (day 3 post transfection) or media was fully exchanged and protein lysates were collected on day 12 post-infection. Statistical comparisons were made by one-way ANOVA plus Hold-Sidak post hoc test. $^{**}p < 0.01$.



Supplemental Figure 10: Time course of HO-1 induction in MDM by tBHQ, MMF, and DMF. Uninfected MDM were exposed to tBHQ (T, 10μM), MMF (M, 30μM), or DMF (D, 30μM) for 0, 6, 12, 24, and 48 hours and assessed for HO-1 expression by Western blot (V = vehicle for tBHQ, MMF, and DMF). For comparison, MDM were also treated with CoPP (CP, 10μM) and SnMP (SP, 10μM) for 24 hours (V2 = vehicle for SnMP and CoPP). (**A**) Representative Western blot of HO-1, NQO1, and GPX expression. Quantification of HO-1 Western blot expression by densitometry analysis normalized to GAPDH for (**B**) tBHQ, (**C**) MMF, and (**D**) DMF treatment. Data points represent mean ± SEM from 6 different MDM donors. Statistical comparisons were made by Student's paired t-test. *$p < 0.05$, **$p < 0.01$.



Supplemental Figure 11: Concurrent treatment with DMF and MMF does not protect neurons in neuroglial cultures from HIV-MDM mediated neurotoxicity. Day 14 in vitro primary rat cerebrocortical neuroglial cultures were exposed to Mock-MDM or HIV-MDM supernatant from day 12 post infection at a 1:20 dilution. HIV-MDM exposed neuroglial cultures were co-treated with increasing doses of **A**) DMF and **B)** MMF. Positive control neuroglial cultures were pre-treated (5 minutes) with either MK801 (a nonselective noncompetitive antagonist of the NMDA receptor) or Ifenprodil (a selective noncompetive antagonist of NR2B subunit-containing NMDA receptors). 24 hours after exposure neuroglial cultures were assessed for MAP2 expression by MAP2 ELISA. MAP2 expression was normalized to untreated neuroglial cultures. Values represent the mean ± SEM from 3 experiments using supernatant from HIV-MDM infections from 3 independent donors. Statistical analysis comparing vehicle treated neuroglial cultures to all other groups were made by one-way ANOVA plus Hold-Sidak post hoc test. ***$p < 0.001$.



Supplemental Figure 12: IFNγ reduces HO-1 protein expression in primary human MDM. MDM were treated with IFNγ (10ng/ml) or Vehicle for 24 hours followed by cell lysis. (**A**) Western blot and (**B**) densitometry quantification of HO-1 expression normalized to GAPDH in MDM following 24 hours of exposure to IFNγ (10ng/ml) in 7 independent MDM donors. Statistical comparison was made by paired Student's t-test. ***p <0.001.

Characteristic	DLPFC Cohort Group			p-value
	HIV -	**HIV+** (No HIVE)	**HIV + HIVE**	
Number of Subjects	66	76	14	-
Age at death, mean ± SD	50.6 ± 16.6	44.4 ± 9.1	41.6 ± 7.1	0.005[a]
Hours postmortem, mean ± SD	14.8 ± 11.2	13.1 ± 14.1	19.7 ± 20.7	0.243[a]
Sex				
Male (%)	49 (74%)	59 (78%)	14 (100%)	0.104[b]
Female (%)	17 (26%)	17 (22%)	0 (0%)	
Race				
White (%)	43 (65%)	48 (63%)	12 (86%)	
Black (%)	16 (24%)	22 (29%)	1 (7%)	0.143[b]
Other/Unknown (%)	7 (10%)	6 (8%)	1 (7%)	
Ethnicity				
Hispanic (%)	19 (29%)	13 (17%)	2 (14%)	0.195[b]
Not Hispanic (%)	41 (71%)	63 (83%)	12 (86%)	
Disease Parameters				
Log plasma HIV c/mL, mean ± SD	-	4.3 ± 1.4	5.2 ± 0.9	0.450[c]
Log CSF HIV c/mL, mean ± SD	-	3.8 ± 0.8	5.9 ± 1.4	< 0.001[c]
Log Brain HIV c/g, mean ± SD	-	2.8 ± 1.1	5.3 ± 1.5	< 0.001[c]
CD4+ lymphocytes/mm^3, mean ± SD	-	116 ± 169	49 ± 49	0.158[c]
Neurocognitive Impairment				
HAND (%)	-	37 (49%)	9 (64%)	0.591[b]
Neuropsych Impairment Other Origin (%)	-	22 (29%)	3 (21%)	
Neurocognitive Normal (%)	-	6 (8%)	0 (0%)	
No Neurocognitive Data (%)	-	11 (14%)	2 (14%)	

Supplemental Table 1 (part 1 of 2)

	DLPFC Cohort Group			
Characteristic	**HIV -**	**HIV+** (No HIVE)	**HIV + HIVE**	**p-value**
ART Treatment Status				
ART-experienced (%)	-	49 (65%)	8 (57%)	
ART-naïve (%)	-	10 (13%)	5 (36%)	0.079[b]
Unknown ART (%)	-	17 (22%)	1 (7%)	

Supplemental Table 1 (part 2 of 2): DLPFC cohort demographic, clinical, and antiretroviral therapy data. Abbreviations: HIV encephalitis (HIVE), standard deviation (SD), HIV-associated neurocognitive disorders (HAND), antiretroviral therapy (ART). Sample size within these group or subgroups are smaller than the overall sample size due the availability of data. [a] Analysis of Variance p-value; [b] Chi-square test p-value ; [c] Student's t-test p-value

Characteristic	Regional Analysis Cohort Group			p-value
	HIV -	**HIV+/HAND** (No HIVE)	**HIV + HIVE**	
Number of Subjects	12	12	10	-
Age at death, mean ± SD	43.0 ± 4.5	41.9 ± 7.6	40.0 ± 5.7	0.518[a]
Hours postmortem, mean ± SD	24.4 ± 7.2	22.2 ± 17.7	26.2 ± 24.9	0.869[a]
Sex				
Male (%)	9 (75%)	10 (83%)	9 (90%)	0.652[b]
Female (%)	3 (25%)	2 (17%)	1 (10%)	
Race				
White (%)	9 (75%)	9 (75%)	8 (80%)	
Black (%)	2 (17%)	1 (8%)	1 (10%)	0.938[b]
Other/Unknown (%)	1 (8%)	2 (17%)	1 (10%)	
Ethnicity				
Hispanic (%)	3 (25%)	1 (8%)	1 (10%)	0.454[b]
Not Hispanic (%)	9 (75%)	11 (92%)	9 (90%)	
Disease Parameters				
Log plasma HIV c/mL, mean ± SD	-	4.5 ± 1.5	5.2 ± 0.9	0.213[c]
Log CSF HIV c/mL, mean ± SD	-	2.8 ± 1.4	5.5 ± 1.4	< 0.001[c]
CD4+ lymphocytes/mm^3, mean ± SD	-	50 ± 106	46 ± 30	0.158[c]
Neurocognitive Impairment				
HAND (%)	-	12 (100%)	6 (60%)	
Neuropsych Impairment Other Origin (%)	-	0 (0%)	3 (30%)	0.053[b]
No Neurocognitive Data (%)	-	0 (0%)	1 (10%)	

Supplemental Table 2: Regional Analysis cohort demographic, clinical, and antiretroviral therapy data. Abbreviations: HIV encephalitis (HIVE), standard deviation (SD), HIV-associated neurocognitive disorders (HAND). Sample size within these group or subgroups are smaller than the overall sample size due the availability of data. [a] Analysis of Variance p-value; [b] Chi-square test p-value ; [c] Student's t-test p-value

Human Gene	Company	Primer and Probe Set Catolog #
CD163	Applied Biosystems	Hs01016661_m1
CD68	Applied Biosystems	Hs00154355_m1
CD8A	Applied Biosystems	Hs01555600_m1
GAPDH	Applied Biosystems	Hs99999905_m1
IRF1	Applied Biosystems	Hs00971959_m1
ISG15	Applied Biosystems	Hs00192713_m1
MX1	Applied Biosystems	Hs00182073_m1

Supplemental Table 3: RT-PCR primer and probe sets.

Primary Antibody	Host Species	Mono- or Polyclonal	Provider	Catlog #	Dilution Used	Final Antibody Concentration	kDa of Band Quantified
β-tubulin	rabbit	monoclonal	Cell Signaling	2128	1:1000	0.014 µg/ml	55 kDa
FTH1	rabbit	polyclonal	Cell Signaling	3998	1:1000	0.071 µg/ml	21 kDa
GAPDH	mouse	monoclonal	Advanced Immunochemical	Mab 6C5	1:30000	0.260 µg/ml	35 kDa
GPX1	rabbit	monoclonal	Cell Signaling	3286	1:1000	0.110 µg/ml	21 kDa
GSTP1	mouse	monoclonal	Cell Signaling	3369	1:1000	0.400 µg/ml	23 kDa
HO-1*	rabbit	polyclonal	Enzo Life Sciences	SPA-895	1:500 - 1:250	2.0 - 4.0 µg/ml	29 kDa
HO-1#	rabbit	polyclonal	Enzo Life Sciences	SPA-896	1:500 - 1:250	2.0 - 4.0 µg/ml	29 kDa
HO-2	rabbit	polyclonal	Enzo Life Sciences	SPA-897	1:500 - 1:250	2.0 - 4.0 µg/ml	35 kDa
NQO1	mouse	monoclonal	Abcam	ab28947	1:1000	1.0 µg/ml	28 kDa
PRDX1	rabbit	polyclonal	Cell Signaling	8732	1:1000	0.096 µg/ml	21 kDa
SOD1	mouse	monoclonal	Cell Signaling	4266	1:1000	0.250 µg/ml	18 kDa
TRXR1	rabbit	polyclonal	Cell Signaling	6925	1:1000	0.025 µg/ml	55 kDa

Supplemental Table 4: Primary antibodies for Western blotting. *Antibody used for Western blot detection of HO-1 in human brain tissue. #Antibody used for Western blot detection of HO-1 in all in vitro studies using primary cells.

Secondary Antibody	Host Species	Provider	Catlog #	Dilution Used	Final Antibody Concentration
IRDye 680RD Goat Anti-Mouse IgG	goat	Licor	926-68070	1:20000	0.050 µg/ml
IRDye 680RD Goat Anti-Rabbit IgG	goat	Licor	926-68071	1:20000	0.050 µg/ml
IRDye 800CW Goat Anti-Mouse IgG	goat	Licor	926-32210	1:15000	0.067 µg/ml
IRDye 800CW Goat Anti-Rabbit IgG	goat	Licor	926-32211	1:15000	0.067 µg/ml
HRP-conjugated AffiniPure Goat Anti-Mouse IgG (H+L)	goat	Jackson Immunoresearch	115-035-003	1:5000	0.160 µg/ml
HRP-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L)	goat	Jackson Immunoresearch	111-035-003	1:5000	0.160 µg/ml
HRP-conjugated Goat Anti-Rabbit IgG (H+L)	goat	Cell Signaling	7074	1:1000	0.071 µg/ml
HRP-conjugated Horse Anti-Mouse IgG (H+L)	horse	Cell Signaling	7076	1:1000	0.111 µg/ml

Supplemental Table 5: Secondary antibodies for Western blotting. Signal intensity was analyzed using ImageJ for HRP-conjugated secondary antibodies and Image Studio (LiCor) for fluorescent secondary antibodies.

Human Gene mRNA Targeted	Company	Product Line	siRNA ID Number
BACH1	Ambion (Life Technologies)	Silencer® Select	s1859*
BACH1	Ambion (Life Technologies)	Silencer® Select	s1860
HO-1	Ambion (Life Technologies)	Silencer® Select	s6673*
HO-1	Ambion (Life Technologies)	Silencer® Select	s6674
HO-2	Ambion (Life Technologies)	Silencer® Select	s6675*
HO-2	Ambion (Life Technologies)	Silencer® Select	s6677
Scramble (Negative Control)	Ambion (Life Technologies)	Silencer® Select	Catalog #: 4390846*

Supplemental Table 6: Small interfering RNAs (siRNAs) used for knockdown experiments. * siRNA used in HIV-MDM experiments'

Supplemental Methods

Subjects in DLPFC cohort

A cohort of 90 HIV-positive (HIV^+) and 66 HIV-negative (HIV^-) subjects was selected from the NNTC (1) autopsy cohort for analysis of protein expression within DLPFC tissue. This cohort was assembled by the Texas NeuroAIDS Research Center and has been described in a prior report (2). The 90 HIV^+ cases included subjects who had pathologically confirmed encephalitis ($HIVE^+$; n=14) and subjects who did not have HIVE ($HIV^+/HIVE^-$; n=76). Among the 76 $HIV^+/HIVE^-$ individuals were 37 confirmed cases of HAND, 6 cases confirmed as neurocognitively normal, and 33 cases with either no associated neurocognitive diagnosis or neurocognitive dysfunction in which factors other than HIV infection were not ruled out. Among the 14 $HIVE^+$ cases, 9 were confirmed to have HAND (the other 5 had either no associated neurocognitive diagnosis or neurocognitive dysfunction in which factors other than HIV infection were not ruled out). Following administration of the neurocognitive test battery implemented by the NNTC (3), the neurocognitive diagnosis of HAND was assigned by the supervising neuropsychologist according to the Frascati Criteria (4) as reviewed by an experienced neurologist. NNTC site neuropathologists rendered nosological diagnoses of HIVE as guided by the criteria of Budka et al (5). Sixty-three percent of HIV^+ subjects were ART-experienced. HIV^- subjects were significantly older than HIV^+ subjects on average by 6.6 years, but did not significantly differ from HIV^+ subjects in gender, ethnicity, race, or post-mortem interval. See Supplemental Table 1 for summarized demographic and clinical data. Further details on the demographic and clinical data of this cohort were described previously (2).

Subjects in regional analysis cohort

A cohort of 12 HIV-negative (HIV^-), 12 HIV-positive with a diagnosis of HAND (HIV^+/HAND), and 10 HIV-positive with HIVE ($HIVE^+$) subjects was selected from the

NNTC (1) for a regional brain analysis of protein expression within the striatum (head of caudate), occipital cortex, and cerebellum (anterior cerebellar cortex). This cohort was matched for age, post mortem interval, sex, race, and ethnicity (Supplemental Table 2). Of the 34 cases in this regional analysis cohort, 16 were represented in the larger DLPFC cohort.

Brain dissection and extraction of protein and RNA

DLPFC was dissected from Brodmann areas 9 or 10. For protein extraction, fresh-frozen brain (30-500 mg) was homogenized by silica bead beating, sonicated, and solubilized (0.03% TX-100, 0.5 mM DTT, 5 mM MgCl2, Tris 10 mM, pH 7.8). RNA was prepared using the RNeasy Lipid Tissue Mini Kit (Qiagen) as previously described (2, 6). Briefly, 100 mg of brain tissue from an adjacent region of protein extraction was dissected on dry ice and homogenized in a minibead beater. RNA was extracted with chloroform and centrifuged in RNeasy mini spin columns, washed, and eluted.

Quantification of viral loads and CD4 count

In the majority of cases, blood and CSF samples were obtained within 6 months of subject death and on the same day that neurocognitive domains were assessed. Plasma and CSF viral loads were determined with the Amplicor HIV-1 Monitor test v1.1 through v1.5 (*Roche*). Brain parenchyma HIV RNA was quantified from brain extracted RNA as previously described (2, 6). Briefly, 1µg microgram of brain RNA and 1µM of antisense primer 84R were used in 20µl reaction (iScript cDNA Synthesis Kit, Bio-Rad Laboratories). Four microliters of cDNA was used for 25µl real-time PCR by using JumpStart Taq ReadyMix for Quantitative PCR (Sigma) and SmartCycler (Cepheid). Results were standardized against a known brain secondary standard. CD4+ T-lymphocyte counts were determined by flow cytometry and performed at each NNTC site's Clinical Laboratory Improvement Amendments (CLIA)–certified, or CLIA equivalent, medical center laboratory.

Quantification of brain mRNAs

Expression of brain mRNAs was quantified after cDNA synthesis from mRNA samples using Taq-Man Universal PCR Master Mix (Applied Biosystems) by RT-PCR as previously described (6). Duplicate RT-PCR reactions were run and relative mRNA expression was calculated using the $\Delta\Delta C_t$ method (compared to GAPDH mRNA expression) using the primer and probe mixes listed in Supplemental Table 3.

Western blot analysis

Cell cultures were rinsed with ice-cold PBS, lysed in 75mM Tris-HCL (pH 6.8) containing 15% glycerol, 3.75mM EDTA, and 3% SDS, and supplemented with Complete Protease Inhibitor Cocktail (Roche) and PhosSTOP phosphatase inhibitor mixture (Roche). Cell and DLPFC protein lysates were subjected to SDS-PAGE as previously described (7) using primary and secondary antibodies listed in Supplemental Tables 4 and 5. For all autopsy cases, protein expression as determined by Western blotting was normalized to both β-tubulin and GAPDH expression (supplemental figures).

Isolation and culture of human monocyte-derived macrophages (MDM)

Human monocytes were isolated from healthy donors by Ficoll density gradient centrifugation as previously described (8). Monocytes were plated at 10.5×10^3 cells/cm^2 in Cell-Bind plates (Corning) and cultured in DMEM supplemented with 10% FBS (Thermo Scientific), 10% horse serum (Invitrogen), 1% nonessential amino acids (Invitrogen), 2mM glutamine (Invitrogen), and 50U/ml penicillin/streptomycin at 37^{o}C, 6% CO_2. Cells were cultured for 7 days in vitro (DIV) and visually inspected for MDM differentiation before use in HIV-infection experiments. MDM were cultured for 7-10 DIV before use in noninfectious experiments.

HIV infection of MDM

Differentiated MDM were exposed to 50-100ng of p24 of HIV-1 89.6 (R5/X4 strain) or Jago (R5 strain) per 10^6 cells for 24 hours. HIV-Jago is a macrophage-tropic CSF isolate from a patient with confirmed HIV-associated dementia (9). Virus stocks were prepared by the University of Pennsylvania Center for AIDS Research Virology Core. Supernatants from HIV-infected or noninfected (mock) MDM were collected every 3 days and stored at -80^{o}C. Supernatants were monitored for HIV replication by quantifying viral reverse transcriptase (RT) activity, as analyzed by the amount of radiolabeled deoxythymidine incorporation (10). Briefly, 10µl of MDM supernatant is incubated with 50µl of RT cocktail (50µM Tris pH 7.8, 75µM KCl, 5µM $MgCl_2$, 0.05% NP-40, 2µM DTT, 5µg/ml, 1.6 mU *poly*(*rA*)·*p*$(dT)_{12\text{-}18}$, and 10µCi/ml dTTP [α-^{32}P]) at 37^{o}C overnight. 30µl of sample is dotted onto DE81 Whatman ion exchange cellular chromatography paper (Fisher) and air dried for 30 minutes, washed four times with 2X UltraPure SCC (Invitrogen), washed once in absolute ethanol, and dried at 80-100^{o}C for 30 minutes. Whatman paper is then placed in scintillation vial with 5ml of Scintiverse BD cocktail (Fisher) and counted for ^{32}P in a scintillation counter.

MDM-mediated neurotoxicity

Rat cerebrocortical mixed neuroglial cultures (~70% neurons, 30% glia) were prepared from E17 embryos of Sprague-Dawley rats, as previously described (7). Cells were plated in tissue-culture plates pre-coated with poly-L-lysine (Peptides International) and maintained in neurobasal media plus B27 supplement (Invitrogen) at 37^{o}C and 5% CO_2. After 7 DIV, approximately one-half volume of fresh media was added to the cells to replace evaporation losses. All cultures were used between days 14 and 17 DIV. Cell-based MAP2 ELISAs were performed on primary rat cerebrocortical cells plated at a density of $1x10^4$ cells/well in 96-well plates as previously described (7, 8). Following a 24 hour exposure to HIV-MDM supernatant (n = 6 technical replicates), neuronal cultures were fixed and fluorescently labeled using the following reagents: mouse anti-

MAP2 (Covance), goat anti-mouse β-lactamase TEM-1 conjugate (Invitrogen), and Fluorocillin Green substrate (Invitrogen). Fluorescence intensity was measured using a flour metric plate reader with a 480/520-nm filter set. MDM supernatant was applied at 1:10-1:60 dilution; the dilution that gave values within the linear range of the assay is presented. For uninfected MDM experiments pure neuronal cultures (~97-99% neurons, $5x10^4$ cells/well) were used in order to detect lower levels of neurotoxicity. To obtain pure neuronal cultures cells were treated with 10μM arabinosylcytosine 48 hours after plating and otherwise handled as described above. Neuronal survival was expressed as a percentage of untreated (UT) cultures. In previous publications we have demonstrated that this MAP-2 ELISA quantification robustly correlates with neuronal death as determined by hand counts of surviving MAP-2 stained neurons in this culture system (11, 12).

MDM extracellular glutamate

Glutamate concentration in MDM supernatant was assayed in triplicate using the Amplex Red Glutamic Acid/Glutamate Oxidase Assay Kit (Invitrogen) according to the manufacturer's directions.

MDM cytotoxicity

Freshly isolated human monocytes were plated at a density of 3.4×10^4 cells/well in Cell-bind 96-well plates. On day 12 of HIV-infection or day 6 of drug treatment, MDM cell cytotoxicity was assayed using the CytoTox-Glo Cytotoxicity Assay (Promega) according to the manufacturer's directions.

siRNA knockdown

Silencer® Select siRNAs (Ambion; Supplemental Table 6) were transfected at a final concentration of 50nM using Lipofectamine RNAiMax (Invitrogen). Lipofectamine and

siRNA were pre-incubated for 10 minutes in 50µl of Opti-Mem (Invitrogen) prior to treatment of MDM. All knockdown experiments were verified by Western blot. Transfection efficiency was evaluated using the BLOCK-IT™ Alexa Fluor® Red Fluorescent Oligo (Invitrogen).

Human Fetal Astrocytes (HFA)

HFA cultures were prepared from fetal brain tissue (gestational age 16-18 weeks) obtained from elective abortion procedures. Tissues were digested with 0.25% trypsin and 10U/mL DNASE I and further dissociated to obtain single-cell suspensions. Cells were maintained under 10% CO_2 for 5 days in mixed glial growth media (DMEM:F12, 10% FBS, 50µg/ml gentamicin, 2µM L-glutamine, 10µg/ml insulin, and 5µg/ml fungizone). Monolayers that remained after shaking cells for 14-18 hours at 200rpm constituted astrocytes (confirmed by *glial fibrillary acidic protein (GFAP)* and glutamate aspartate transporter 1 (GLAST-1) staining) and were fed with astrocyte growth media **(**DMEM:F12, 15% FBS, 50µg/ml gentamicin, 2µM L-glutamine, 10µg/ml insulin, and 5µg/ml fungizone) and plated to 6 or 12 well plates for experiments. For 24 hour experiments, cells were treated on DIV6 and collected on DIV7. For 15 day experiments, treatment began on DIV2, media and treatment was replaced every 3 days, and cells were collected on DIV17.

Drugs and chemical preparations

Stock solutions of DMF, MMF, tBHQ, and camptothecin (Sigma-Aldrich) were prepared in DMSO and stored at -20^{o}C until use. CoPP and SnMP (Frontier Scientific) were prepared in 1N NaOH and stored at -20^{o}C until use. Stock solutions of mercaptosuccinic acid and dicoumarol (Sigma-Aldrich) and IFNγ, TNFα, and LPS (Peprotech) were prepared in PBS and stored at -20^{o}C until use. Stock solutions of efavirenz (NIH AIDS Research and Reference Reagent Program) were prepared in DMSO and frozen at -80^{o}C until use.

Experimental Blinding

All studies of protein and RNA brain expression were performed with coded samples by a blinded investigator. For all cell culture studies, the investigator performed treatments, cell harvesting, and Western blotting according to standard protocols without blinding. Quantification of neurotoxicity by MAP-2 ELISA and quantification of HIV replication by reverse transcriptase assay were performed by investigators blinded to treatment conditions.

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