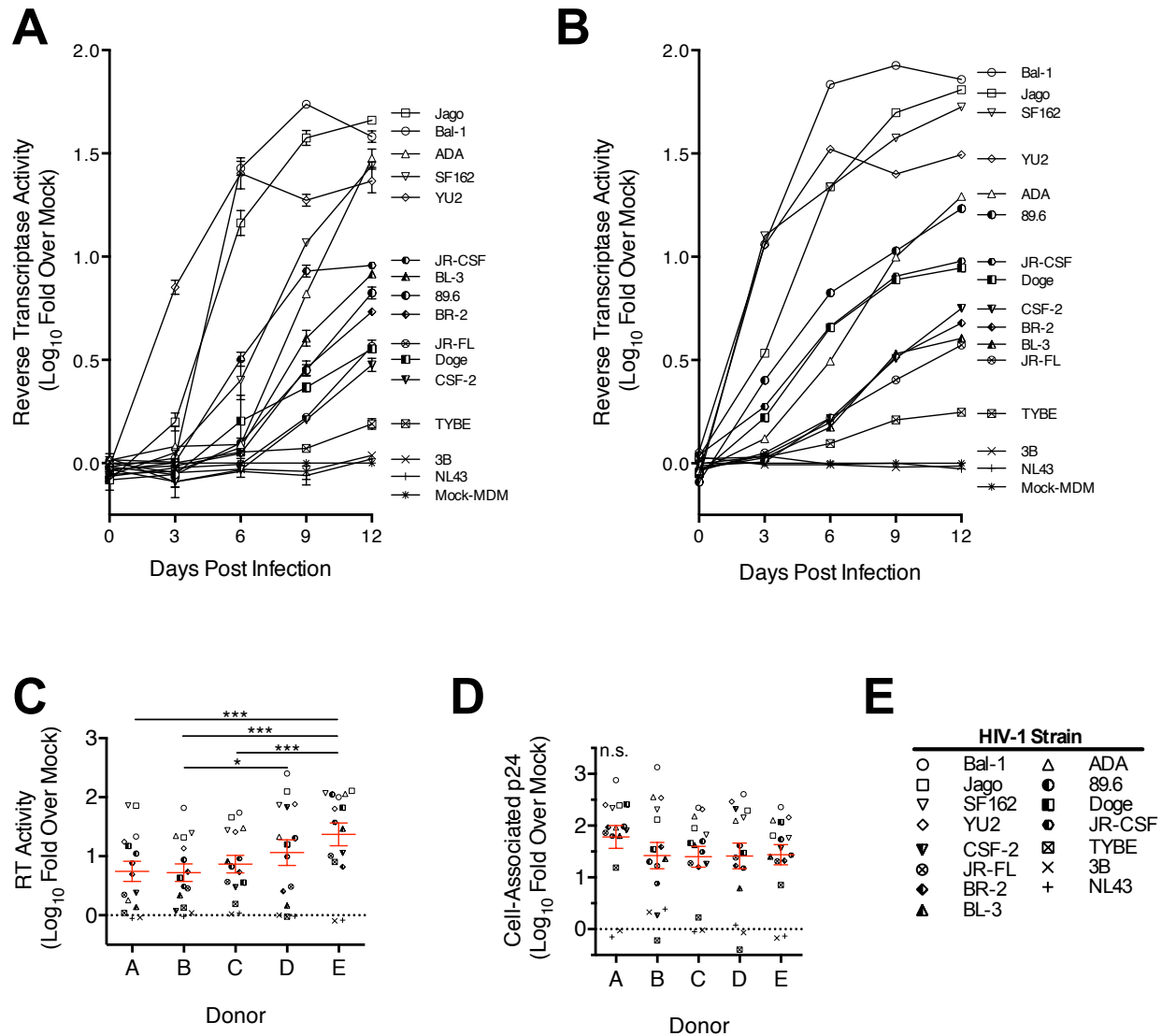
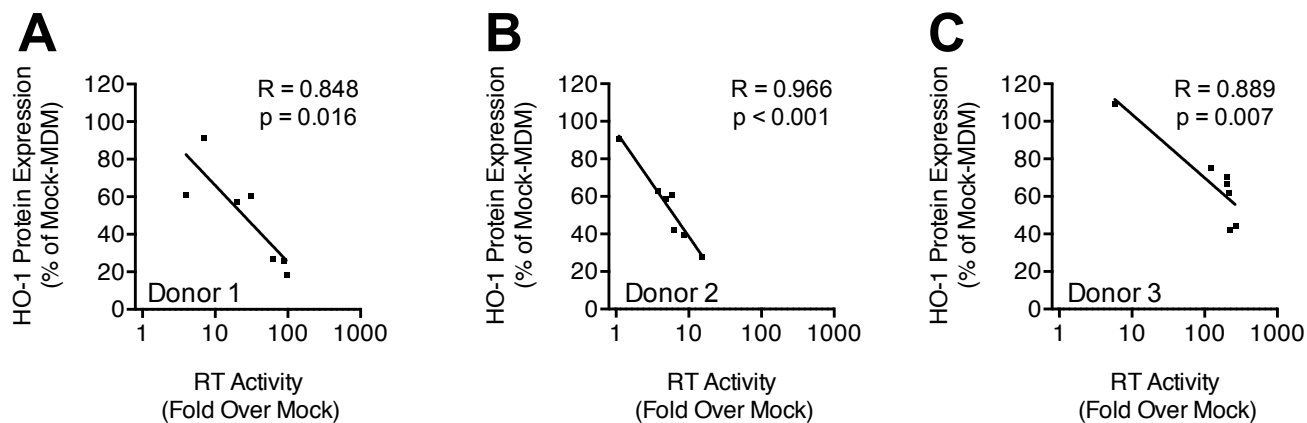


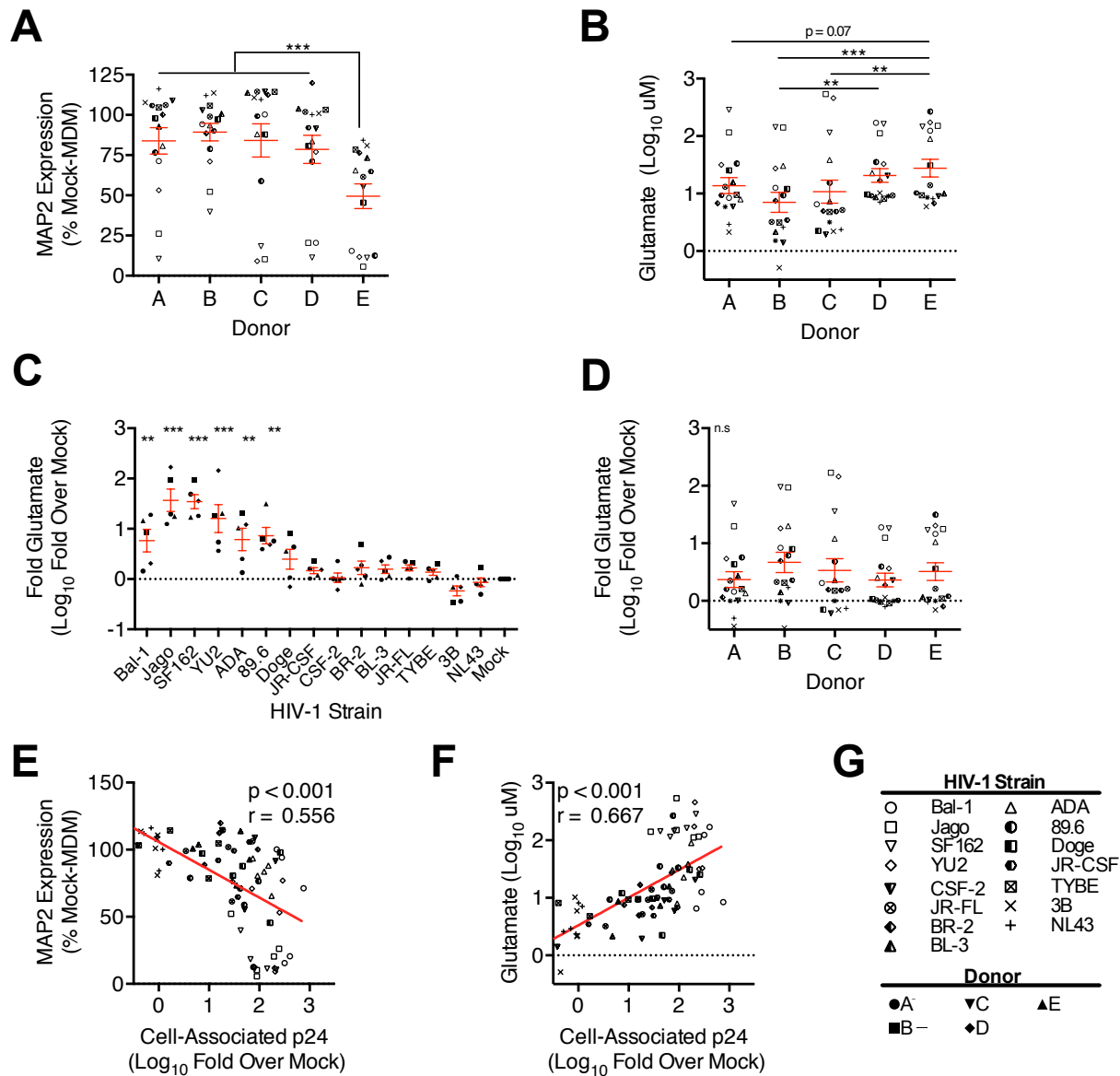
# Supplemental Material



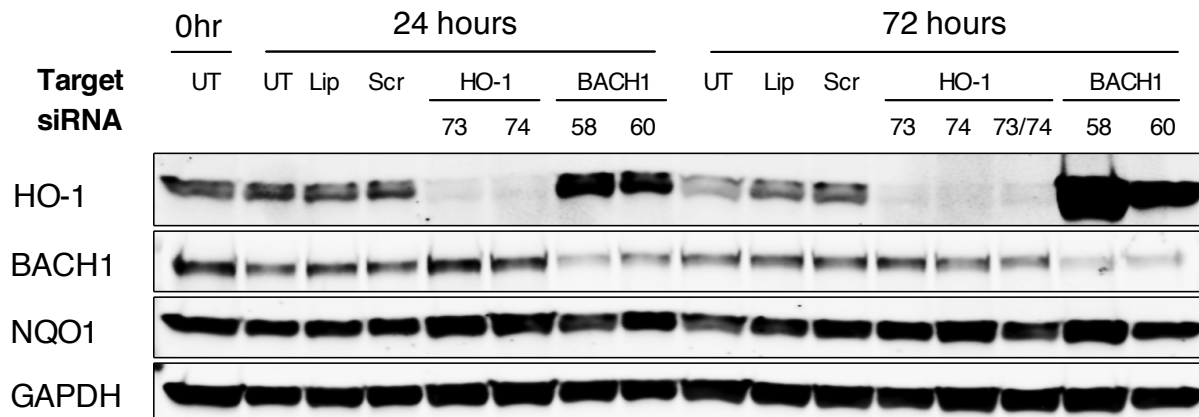
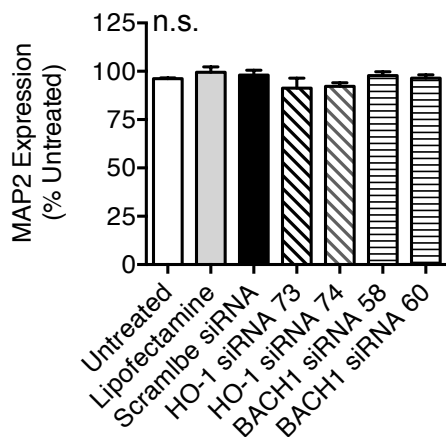
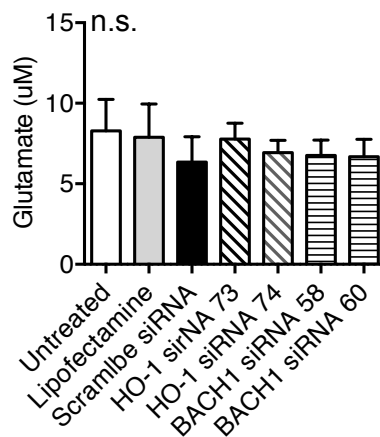
**Supplemental Figure 1: HIV-1 strain replication in primary human macrophages from different donors.** Replication of fifteen HIV-1 viral strains (inoculum 25ng p24 per 400,000 cells) in monocyte-derived macrophages (MDM) isolated from five healthy donors was assessed every 3 days over 12 days of infection by supernatant reverse transcriptase (RT) activity. **A**) Supernatant RT activity in Donor C over the course of infection normalized to Mock-MDM background. Values represent mean  $\pm$  SEM. **B**) Average supernatant RT activity in all five donors over the course of infection normalized to Mock-MDM background. Errors bars are not shown for clarity. **C**) Day 12 supernatant RT activity in all 5 donors stratified by donor normalized to uninfected/ Mock macrophages. **D**) Day 12 HIV-MDM lysate p24 content in all five donors stratified by donor as assessed by Western blot. **E**) Key for HIV strain symbols. Error bars indicate mean  $\pm$  SEM. Statistical comparisons to between donors were made by two-way ANOVA with Holm-Sidak post hoc test. \*  $p < 0.05$ , \*\*\* $p < 0.001$



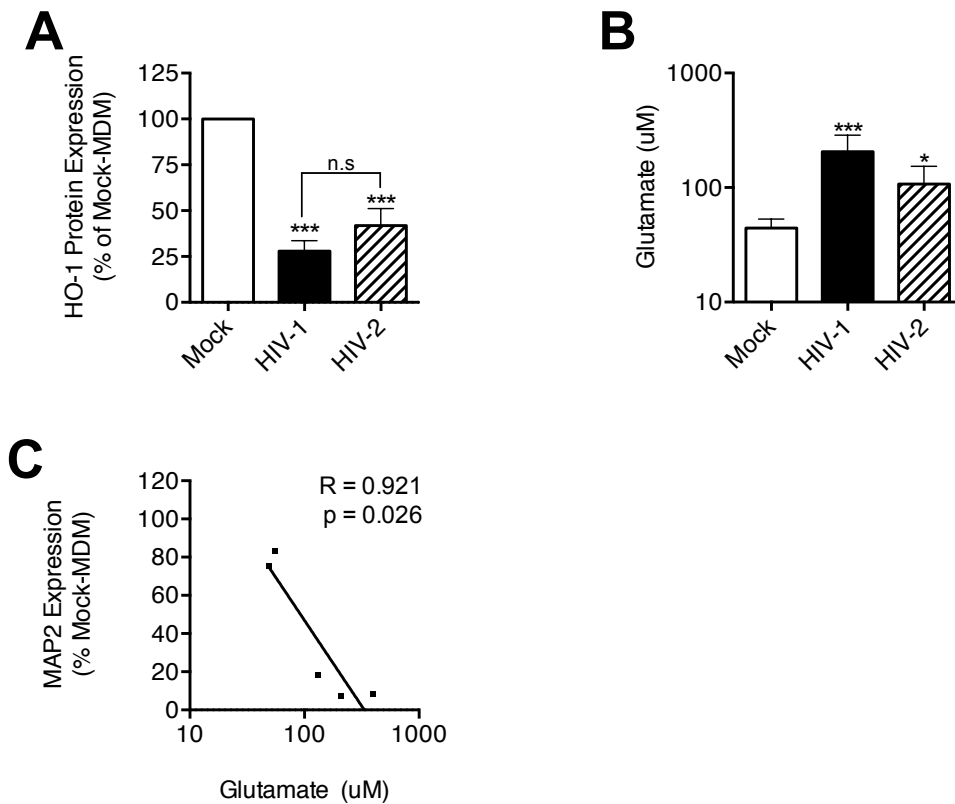
**Supplemental Figure 2: Replication level of an HIV-1 strain in different donor MDM predicts HO-1 protein loss.** MDM from three independent donors were infected with a range of inoculum (0.2 to 50ng/ml HIV p24 per  $4 \times 10^5$  cells) and viral replication by supernatant RT activity and MDM HO-1 protein expression normalized to GAPDH by Western blot were determined. **A-C)** Correlation between day 12 post infection HO-1 protein expression and RT activity for each donor. Correlations were assessed by Pearson's correlation with line of best fit determined by linear regression



**Supplemental Figure 3: HIV replication in macrophages increases extracellular glutamate and associated neurotoxicity.** Neurotoxicity of MDM supernatants was assessed on day 12 post HIV infection by quantification of total microtubule associated protein-2 (MAP2) expression in a cell-based ELISA where primary rat cortical neurons are exposed to HIV-MDM supernatants (1:20 dilution). Glutamate levels were quantified in the same supernatants by Amplex Red Glutamate Assay. **A)** Supernatant neurotoxicity as measured by MAP2 ELISA normalized to Mock-MDM and **B)** supernatant glutamate concentration across 15 HIV-1 strains stratified by donor. Fold supernatant glutamate levels in 5 donors stratified **C)** by strain and **D)** by donor normalized to Mock-MDM supernatant glutamate. Correlation between cell-associated HIV p24 protein expression as measured by Western blot (normalized to GAPDH) and supernatant **E)** neurotoxicity as measured by MAP2 ELISA and **F)** glutamate concentration. **G)** Key for HIV strain and MDM donor symbols. Error bars indicate mean  $\pm$  SEM. Statistical comparisons to Mock-MDM and between donors were made by two-way ANOVA with Holm-Sidak post hoc test. Correlations were assessed by Pearson's correlation with line of best fit determined by linear regression. \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

**A****B****C**

**Supplemental Figure 4: Targeted siRNA knockdown of HO-1 or the HO-1 repressor BACH1 in uninfected MDM does not alter supernatant neurotoxicity or glutamate content.** MDM from 4 independent donors were transfected using Lipofectamine RNAiMax with 50nM of siRNA targeting either HO-1 or BACH1. Two distinct Silencer Select (Ambion) siRNAs targeting HO-1, s6673 (73) and s6674 (74), or BACH1, s1859 (59) and s1860 (60), were used independently to efficiently knockdown or derepress HO-1, respectively. Additional MDM were either left untreated (UT), exposed to lipofectamine (Lip), or transfected with scramble siRNA (Scr; #4390856 Ambion). **A**) Representative Western blot for HO-1, BACH1, NQO1, and GAPDH at 24 hours and 72 hours post transfection with select siRNAs. MDM supernatant **B**) neurotoxicity as measured by MAP2 ELISA and **C**) glutamate content 72 hours post transfection. Errors bars indicate mean  $\pm$  SEM (n = 4). Statistical comparisons to Mock-MDM were made by one-way ANOVA with Holm-Sidak post hoc test. n.s. = not significant.



**Supplemental Figure 5: HIV-2 replication in MDM induces HO-1 deficiency and associated extracellular glutamate and supernatant neurotoxicity.** MDM from 8 independent donors were infected with either HIV-2 CBL-20 or HIV-1 89.6. Supernatants and MDM lysates were analyzed from day 12 post infection. HIV-2 infection of MDM from 5 of 8 donors demonstrated significant HIV-2 replication above background as determined by supernatant RT activity and were further analyzed. **A**) HO-1 protein expression normalized to GAPDH as determined by Western blot densitometry analysis. **B**) Supernatant glutamate content as determined by Amplex Red Assay. **C**) Correlation between supernatant glutamate content and MAP2 ELISA neurotoxicity from day 12 post infection supernatants from HIV-2 infected MDM. Errors bars indicate mean  $\pm$  SEM (n = 5). Statistical comparisons to Mock-MDM were made by one-way ANOVA with Holm-Sidak post hoc test. Correlations were assessed by Pearson's correlation with line of best fit determined by linear regression. \*  $p < 0.05$ , \*\*\*  $p < 0.001$

Donor/Source	Strain	Swarm v. Molecular Clone	Co-receptor Usage	Tissue Isolated From	Clinical Diagnoses	Reference
A	Jago	Swarm	CCR5	CSF	AIDS Dementia Complex Stage 0.5	(1)
B	Doge	Swarm	CCR5	CSF	AIDS Dementia Complex Stage 1	(1)
C	TYBE	Swarm	CXCR4	CSF	CMV Encephalitis	(1, 2)
D	BR-2	Swarm	CCR5	Brain	Progressive Dementia	(3, 4)
D	CSF-2	Swarm	CCR5	CSF	Progressive Dementia	(3, 4)
E	BL-3	Swarm	CXCR4	PBMC		(5)
F	SF162	Swarm	CCR5	CSF	Toxoplasmosis, Acute Meningitis	(6, 7)
G	JR-FL	Swarm	CCR5	Frontal Lobe	AIDS Encephalopathy, Kaposi's Sarcoma	(8, 9)
G	JR-CSF	Swarm	CCR5	CSF	AIDS Encephalopathy, Kaposi's Sarcoma	(8, 9)
H	89.6	Clone	CCR5/ CXCR4	Blood	AIDS with no neurological disease	(9, 10)
I	NL43	Clone	CXCR4	Blood/ bone marrow	AIDS and non-AIDS	(11)
I	3B	Swarm	CXCR4	Blood/ bone marrow	AIDS and non-AIDS	(12, 13)
J	YU2	Clone	CCR5	Brain	HIV-1 Associated Encephalopathy	(14)
K	Bal-1	Swarm	CCR5	Lung	Pediatric patient with AIDS	(15)
L	ADA	Swarm	CCR5	Blood	Kaposi's Sarcoma	(16)

**Supplemental Table 1:** Characteristics of select HIV-1 strains provided by the Center for AIDS Research Virology Core at the Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA.

Primary Antibody	Host Species	Mono- or Polyclonal	Provider	Catlog #	Dilution Used	Final Antibody Concentration	kDa of Band Quantified
GAPDH	mouse	monoclonal	Advanced Immunochemical	Mab 6C5	1:30000	0.260 µg/ml	35 kDa
HIV1 p24	mouse	monoclonal	Abcam	ab9071	1:1000	1.35 µg/ml	24 kDa
HO-1 <sup>#</sup>	rabbit	polyclonal	Enzo Life Sciences	SPA-896	1:500	2.0 µg/ml	29 kDa
HO-2	rabbit	polyclonal	Enzo Life Sciences	SPA-897	1:500	2.0 µg/ml	35 kDa

**Supplemental Table 2:** Primary antibodies for western blotting.

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

**Supplemental Table 3:** Secondary antibodies for Western blotting.

Human Gene	Company	Primer and Probe Set Catalog #
GAPDH	Applied Biosystems	Hs0275899_g1
HMOX1 (HO-1)	Applied Biosystems	Hs01110250_m1
HMOX2 (HO-2)	Applied Biosystems	Hs00909233_m1

**Supplemental Table 4:** RT-PCR Primer and Probe Sets

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