Supplemental Material

Figure legends

Figure 5: HIV-infection of astrocytes induces apoptosis in uninfected astrocytes by a gap junction dependent mechanism. (A) Quantification of apoptosis was performed by double labeling with TUNEL and GFAP staining. HIV-infection of astrocyte cultures with HIV_{ADA}, HIV_{JR-CSF}, or $HIV_{92UG021}$ resulted in significantly increased apoptosis after 7, 14 and 21 days post infection (*$p<0.005$, n=15). (B) Whether HIV_{ADA}, HIV_{JR-CSF} or $HIV_{92UG021}$ infection alters GJ communication was evaluated, by a scrape loading technique. HIV-infection reduced GJ communication ~20-40% as compared to uninfected astrocytes (100 %), but the GJ still remained functional (*$p<0.003$, n=11). (C) To evaluate whether GJ communication participates in apoptosis of uninfected astrocytes in contact with HIV-infected astrocytes, gap junction blockers were used. Addition of 18-α-glycyrrhetinic acid (AGA; 50 μM) or octanol (500 μM) after 1, 2 and 3 days post-infection reduced GJ communication by ~70 % as compared to HIV-infected astrocytes cultures (# $p<0.002$; n=17). Similar results were found using octanol (data not shown). (D) Blocking gap junction channels with AGA treatment resulted in significant reduction in astrocyte apoptosis mediated through HIV-infected cells, # $p<0.003$; n=17. These results demonstrate that GJ communications mediate transfer of pro-apoptotic signals between HIV-infected astrocytes and uninfected astrocytes that form GJ with infected cells. Gray diamonds (♦) represents the values of apoptosis shown by HIV-infected cultures without AGA (results shown in A), for comparison of apoptosis levels with AGA (E, # $p<0.0002$; n=17).

Figure 6: HIV-infection of astrocytes alters glutamate metabolism and CCL2 secretion by a gap junction dependent mechanism. To examine the functional consequences of HIV-

infection in astrocytes and the role of gap junctions in control extracellular levels of glutamate and CCL2, quantification of extracellular levels of glutamate and CCL2 was performed by ELISA. (A) Extracellular levels of glutamate from uninfected astrocytes were stable during the time course examined (■), HIV-infection of cultures of astrocytes with ADA (●), JR-CSF (▲) or 92UG021 (▼) viruses resulted in a time dependent accumulation of extracellular glutamate in the media as compared to control conditions (* $p<0.0002$; n=9). The gap junction blocker, AGA, reduced the increase in extracellular glutamate triggered by HIV-infection (# $p<0.0005$ as compared to viral infection alone; n=9). (B) CCL2 secretion was highly increased by HIV-infection and addition of AGA to HIV-infected cultures resulted in ~50% reduction in the amount of CCL2 secretion induced by HIV-infection alone (*$p<0.0002$ as compared to control conditions, and # $p<0.0002$ as compared to HIV-infection alone; n=9). These results indicate that HIV-infection of just a few astrocytes results in dysregulation of glutamate metabolism and enhanced secretion of CCL2 into the extracellular medium by a gap junction dependent mechanism.