Supplemental Figures

Supplemental Figure 1: Trafficking of VSV-G pseudotyped lentiviral vectors in NSC-34 cells.

NSC-34 cells were incubated with VSV-G pseudotyped HIV-1 particles labelled with Vybrant DiD (MOI 10) for 10 minutes before being imaged by time lapse confocal microscopy. Graph shows the displacement of VSV-G pseudotyped HIV1 particles (104 tracks).

Supplemental Figure 2: Trafficking of FlAsH-labelled RV-G pseudotyped HIV-1 particles in NSC-34 cells.

Differentiated NSC-34 cells were incubated with FlAsH labelled RV-G pseudotyped HIV-1 particles for 10 minutes (MOI 10) before imaging. A) Representative time series from supplemental movie 2, showing retrograde trafficking particles (red and yellow circles, cell body is on the left). B) The same time series represented as kymograph. C) Single vector analysis showing the displacement from the origin of each particle analysed (17 tracks). D) Speed distribution analysis of individual particle movements (speed between frames), 488 individual events.

Supplemental Figure 3: Comparison of retrograde speed distribution of RV-G pseudotyped HIV-1 vectors in NSC-34 cells and primary motor neurons.

Single particle speed analysis was performed on retrograde trafficking particles. A) Cumulative frequency profile of the speed of individual movements (between each frame) in motor neurones (red, Fig.3) and differentiated NSC-34 cells (blue, Fig. 3). B) Speed distribution profile of the same data.

Supplemental figure 4: Trafficking of RV-G pseudotyped lentiviral vectors labelled with FlAsH in primary motor neurons.

A-D) Motor Neurons (DIV 3) were incubated with FlAsH labelled RV-G pseudotyped HIV-1 vectors for 30 mins (MOI 10) before time-lapse confocal imaging. A) Representative image series of trafficking vector particles from Supplemental movie 4. B) Kymograph of the same image series. C) Individual track analysis and D) Speed distribution analysis of moving particles (>20 µm in one direction, 20 tracks, 458 individual events)

Supplemental Figure 5: Many vectors and $p75^{NTR}$-positive endosomes do not undergo endosomal trafficking.

Representative kymograph of DiO labelled RV-G pseudotyped HIV-1 vectors (i, pseudocoloured in green), and anti-$p75^{NTR}$-AF647 (ii, pseudocoloured in red). Arrows denote vector particles undergoing very little directional movement, but are still colocalised with similarly stationary $p75^{NTR}$ -positive endosomes, in contrast to the typical rapidly moving endosomes (ii).

Supplemental Figure 6: nAChR undergo retrograde trafficking in the same pathway as TeNT.

A and B) Primary motor neurons plated in microfluidics chambers (DIV 7) α-bungarotoxin-AlexaFluor-647 (100 ng/µl final concentration) for 60 minutes in axonal chamber before time-lapse confocal imaging of a region of axon >200 from the axonal compartment. A) Image series of trafficking endosomes. B) Kymograph of the same image series. C-D) Motor neurons (DIV 7) grown in microfluidics chambers were incubated with DiO labelled RV-G psudotyped HIV-1, TeNT-AF555 and α-bungarotoxin-AlexaFluor-647 for 2 hours at $37^{o}C$ before fixing and imaging by confocal

microscopy. Shown are representative maximum projections of Z-stack images (C), where white circles show examples of colocalisation of α-bungarotoxin-AlexaFluor-647 with both vector and TeNT-AF555 and red circles show examples of colocalisation with just TeNT-AF555. E) Shows quantification of colocalisation (n = 3, 323 α-bungarotoxin-AlexaFluor-647 endosomes, 64 RV-G psudotyped DiO labelled vectors).

Supplemental Figure 7: Lipophilic labelling of RVG pseudotyped HIV-1 particles does not affect transduction efficiency of MNs in MFCs.

A, B and C) Primary motor neurons plated in microfluidics chambers (DIV7). A) EGFP expressing RVG pseudotyped HIV1-DID and RVG pseudotyped HIV-1 non-labelled vectors were applied to the somatic chamber for 6 hours at 37˚C. B) EGFP expressing RVG-HIV1-DID and RVG-HIV1-non-labelled vectors were applied to the axonal chamber for 6 hours at 37˚C. (A and B) 72 hours later, motor neurons were fixed, stained with antibodies against EGFP and SMI32 and imaged by confocal microscopy to assess transduction of MNs. Cell bodies are located on the left; axons traversing through the microgrooves and reaching the axonal compartment located on the right. Scale bars, 100 μm.

Supplemental Figure 8: Collage image of a rare transduction of neuron from vector application on the axonal chamber.

Primary motor neurons were plated in microfluidics chambers and EGFP expressing RV-G pseudotyped HIV-1-DiD vectors were applied to the axonal chamber for 6 hours at 37 ˚C. 72 hours post vector addition, the cells were fixed and EGFP expression imaged by confocal microscopy using a 40x objective. Scale bar 100 μm

Supplemental Figure 9: RVG-pseudotyped HIV-1 lentiviral vector motor neuron transduction efficiency following intramuscular delivery.

A. Representative spinal cord section containing Fast Blue retrogradely traced motor neurons (in black) ipsilateral to the site of injection. 44±26.8 total Fast Blue labelled MNs were detected spanning though the 4th and 5th lumbar spinal segment. B. RVG pseudotyped lentiviral vector transduction efficiency plotted as percentage of total retrogradely traced MNs. RVG-pseudotyped lentiviral vector transduced 13±9.64 % of total Fast Blue labelled MNs. C. Representative spinal cord section containing RVG pseudotyped lentiviral vector retrogradely transduced MNs. Scale bar 200 μm. Both the retrograde tracer and the lentiviral vector were injected intra-muscularly in right hindlimb gastrocnemius muscle of mice. Fast Blue retrograde tracer was injected dissolved 2% in water, and the volume delivered was 3 μl over a single injection n=3. RVG pseudotyped LV biological titre was $1.66x10^9$ TU/ml and the volume delivered was 10 μl divided as 2 x 5 μl injections n=3. Spinal cords were harvested 8 days post-injection of Fast Blue and 3 weeks post RVG-pseudotyped lentiviral vector injection respectively.

Supplemental Movies

Supplemental Movie 1: RV-G pseudotyped HIV-1 undergoes retrograde trafficking in NSC-34 cells.

Image series of DiD labelled RV-G pseudotyped HIV-1 particles in differentiated NSC-34 cells. Cell body is on the left. Image is 140 μm in length.

Supplemental Movie 2: RV-G pseudotyped HIV-1 undergoes retrograde trafficking in NSC-34 cells.

Image series of FlAsH labelled RV-G pseudotyped HIV-1 particles in differentiated NSC-34 cells.

Supplemental Movie 3: RV-G pseudotyped HIV-1 undergoes trafficking in primary motor neurons.

Image series of DiD labelled RV-G pseudotyped HIV-1 particles in primary motor neurons. Cell body is on the left, image is 100 µm in length.

Supplemental Movie 4: RV-G pseudotyped HIV-1 undergoes trafficking in primary motor neurons.

Image acquisition series of FlAsH labelled RV-G-HIV-1 particles in primary motor neurons. Cell body is on the left.

Supplemental Movie 5: RV-G pseudotyped HIV-1 vectors undergo retrograde trafficking in $p75^{NTR}$ positive endosomes.

Primary motor neurones were incubated with DiO labelled RV-G psudotyped HIV-1 particles and anti-$p75^{NTR}$-AF647. A) Acquisition series of vector trafficking, with tracking data added in ImageJ (circles). B) Acquisition series of both DiO labelled RV-G psudotyped HIV-1 (green) and $p75^{NTR}$-AF647 (magenta). Again, circles label the trafficking vector particles, image is 125 µm in length.

Supplemental Movie 6: RV-G pseudotyped HIV-1 vectors undergo retrograde trafficking in $p75^{NTR}$ positive endosomes in microfluidic chambers.

Primary motor neurones grown in microfluidics chambers were incubated with DiO labelled RV-G-HIV-1 particles and anti-$p75^{NTR}$-AF647. A) Shows the acquisition series of vector trafficking. B) Shows the same series in green with the $p75^{NTR}$ time series overlaid (magenta). Image is 150 µm length.

Supplemental Movie 7: RV-G targets EIAV vectors for $p75^{NTR}$ endosome mediated transport. Primary motor neurons grown in Matek dishes were incubated with FlAsH labelled RV-G EIAV particles and anti-$p75^{NTR}$-AF647 antibody. A) Shows a time series of vector particle trafficking. B) The same time series is shown in green with $p75^{NTR}$ series overlaid (magenta).