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Supplemental Information

Deletion of the Virion Host Shut-off Gene

Enhances Neuronal-Selective Transgene Expression

from an HSV Vector Lacking Functional IE Genes

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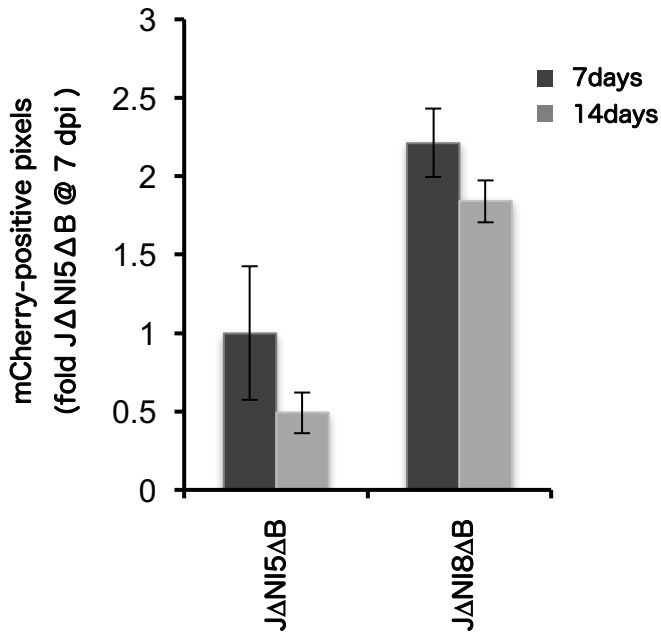


Figure S1. mCherry protein levels in JΔNI5ΔB- and JΔNI8ΔB-infected rDRG cultures. Duplicate wells of cells were infected with each virus as in Figure 2A (3000 gc/cell) and 4 different microscopic fields of each well were photographed at 7 and 14 dpi. The percentage mCherry-positive pixels in each field was determined from the number of pixels above threshold to total pixels established by ImageJ analysis (Fiji, <http://fiji.sc/>). Data are presented relative to JΔNI5ΔB-infected cells at 7 dpi and represent averages \pm SD of two independent experiments.

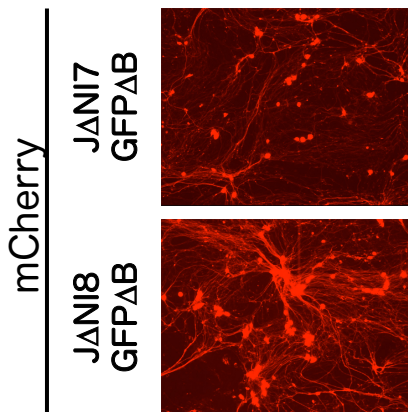


Figure S2. mCherry expression in JΔNI7GFPΔB- and JΔNI8GFPΔB-infected rDRGs. The images document mCherry fluorescence in the same fields as GFP fluorescence shown in Figure 3D (7 dpi).

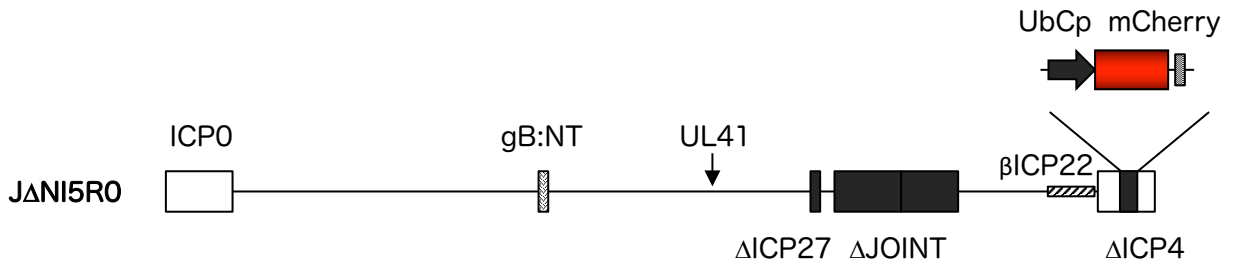
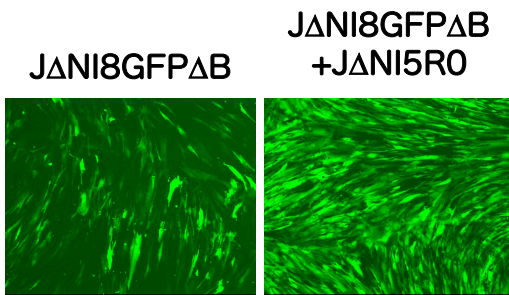
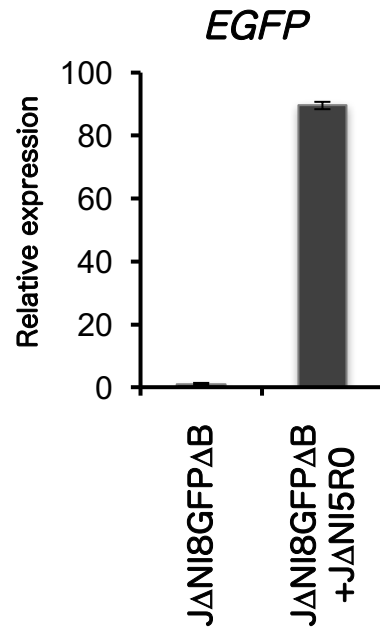
A**B****C**

Figure S3. $J\Delta NI5R0$ genome structure and activity. **(A)** HSV-BAC engineering was used to repair the deleted ICP0 locus in the U_L -flanking terminal repeat of $J\Delta NI5$. **(B)** $J\Delta NI5R0$ superinfection enhances GFP fluorescence in $J\Delta NI8GFP\Delta B$ -infected fibroblasts. HDFs in 96-well plates were infected with $J\Delta NI8GFP\Delta B$ at 25000 gc/cell and superinfected 6 days later with mock or $J\Delta NI5R0$ virus at 10^8 gc/well. GFP fluorescence was imaged 24 h after superinfection. **(C)** Relative GFP mRNA levels in mock- and $J\Delta NI5R0$ -superinfected cells. Following HDF infection and superinfection as above, the cells were harvested 24 h after superinfection for mRNA and DNA isolation. GFP mRNA levels measured by qRT-PCR were normalized to $J\Delta NI8GFP\Delta B$ viral genome copy numbers determined by qPCR for the CAG promoter. Averages \pm SD of two independent experiments.

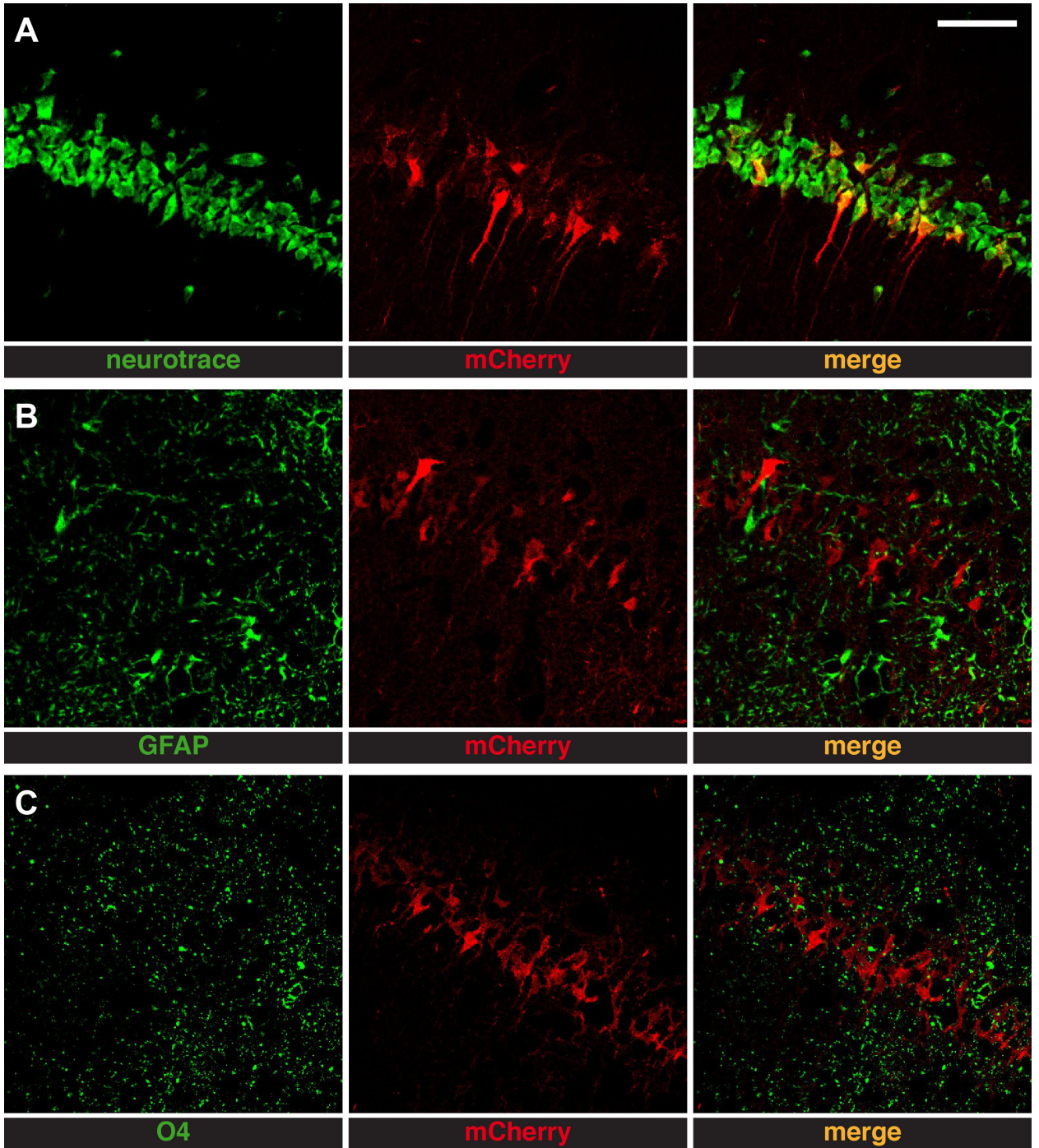


Figure S4. Neurons were the prevalent cell type expressing mCherry after $J\Delta NI8\Delta B$ injection of the hippocampus. Representative confocal images from coronal sections prepared from animals killed 1 week after vector injection into the right hippocampus (4 animals/group). Note overlapping signal in neuronal cell bodies (**A**, NeuroTrace) for all mCherry-expressing cells. No overlap was observed with GFAP, a marker of astrocytes (**B**) or with O4, a marker of oligodendrocytes (**C**). Horizontal bar, 100 μm .