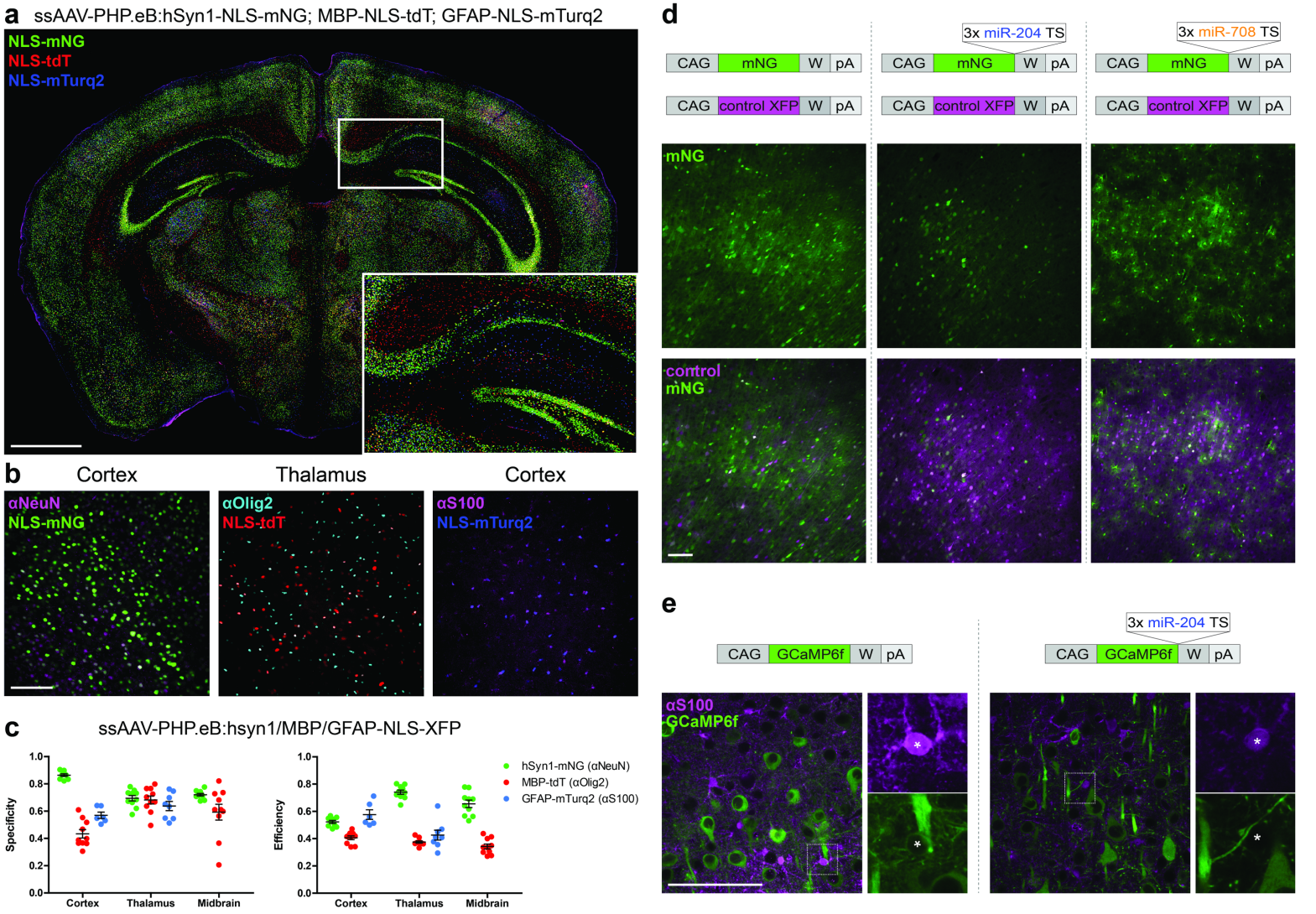
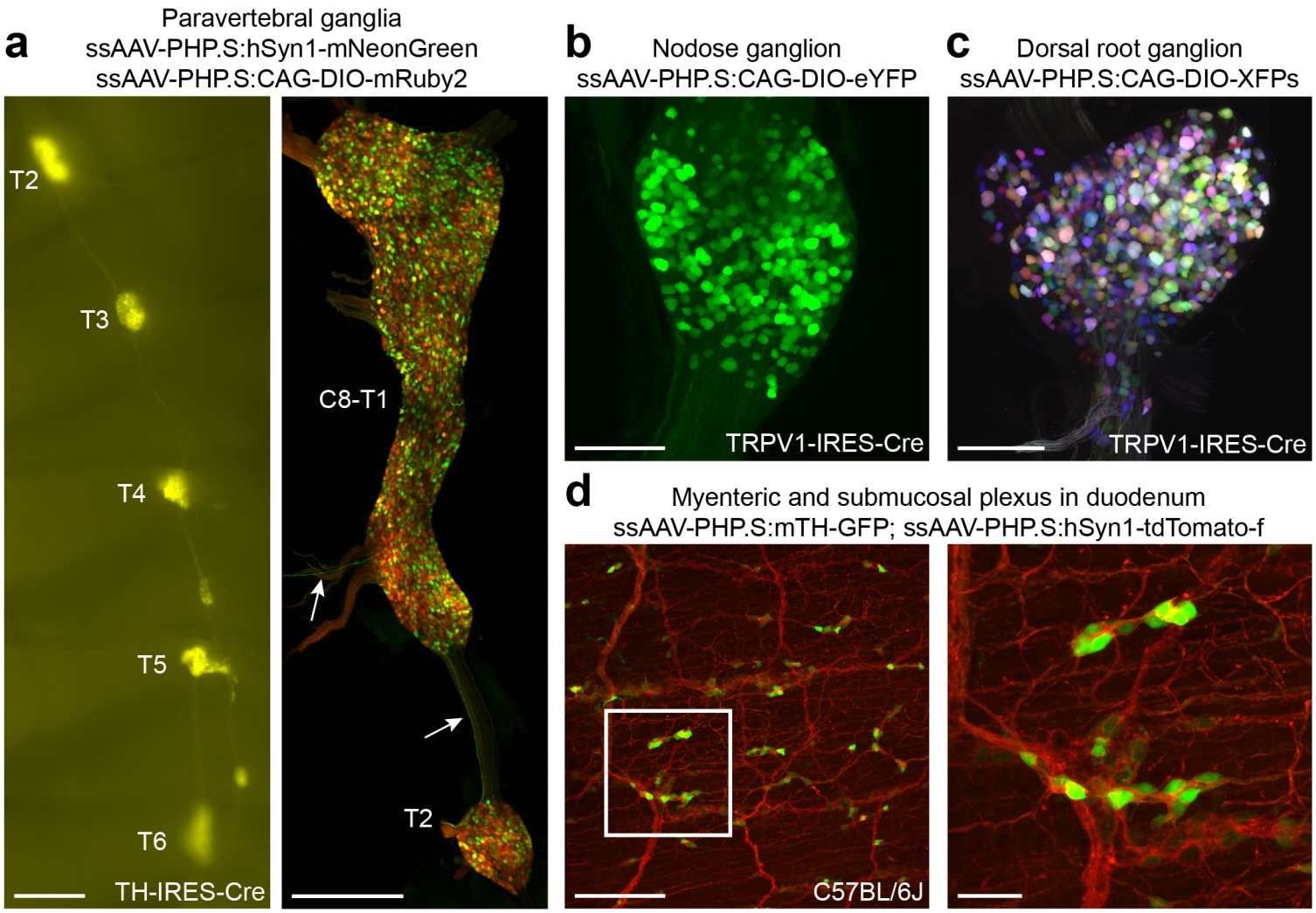
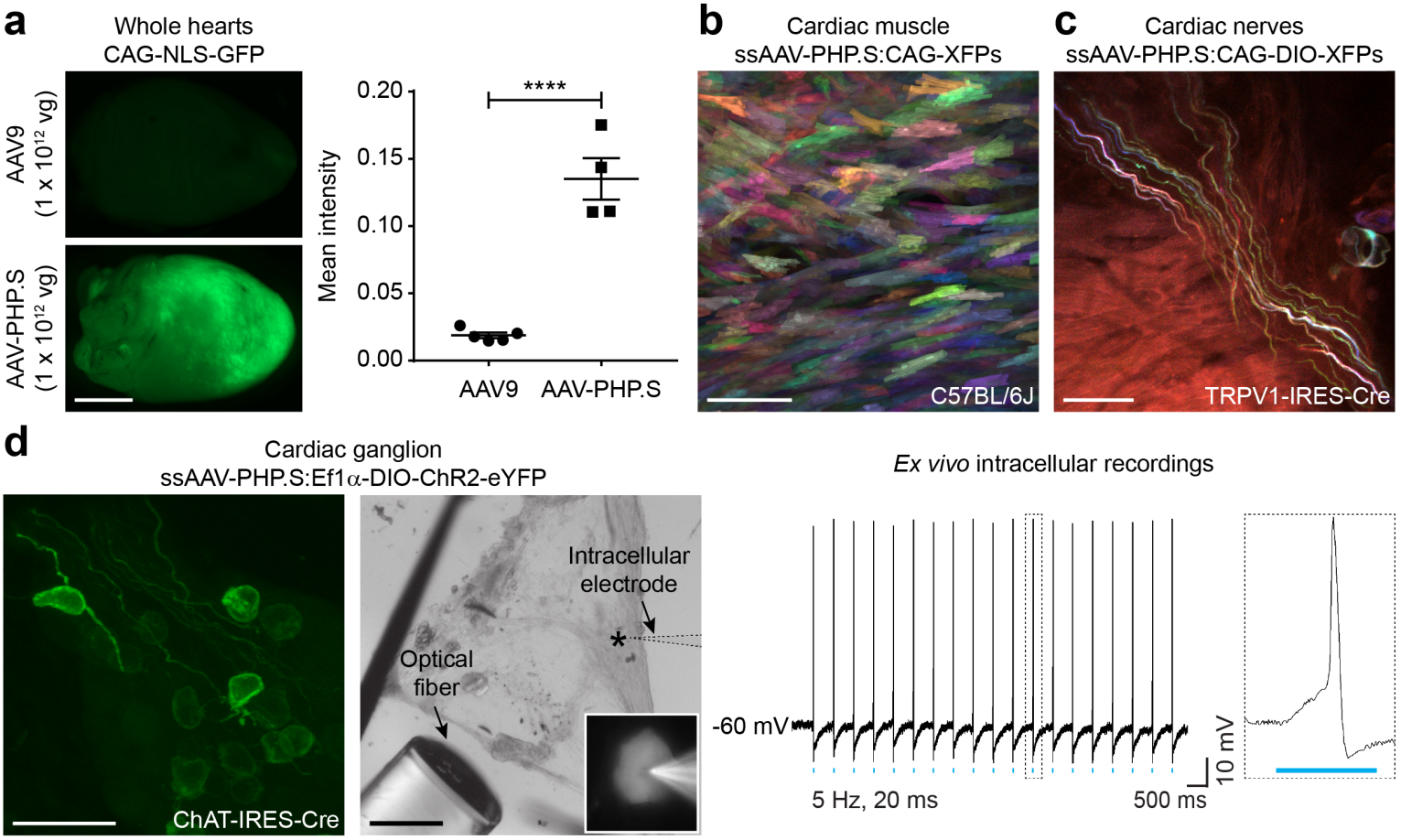


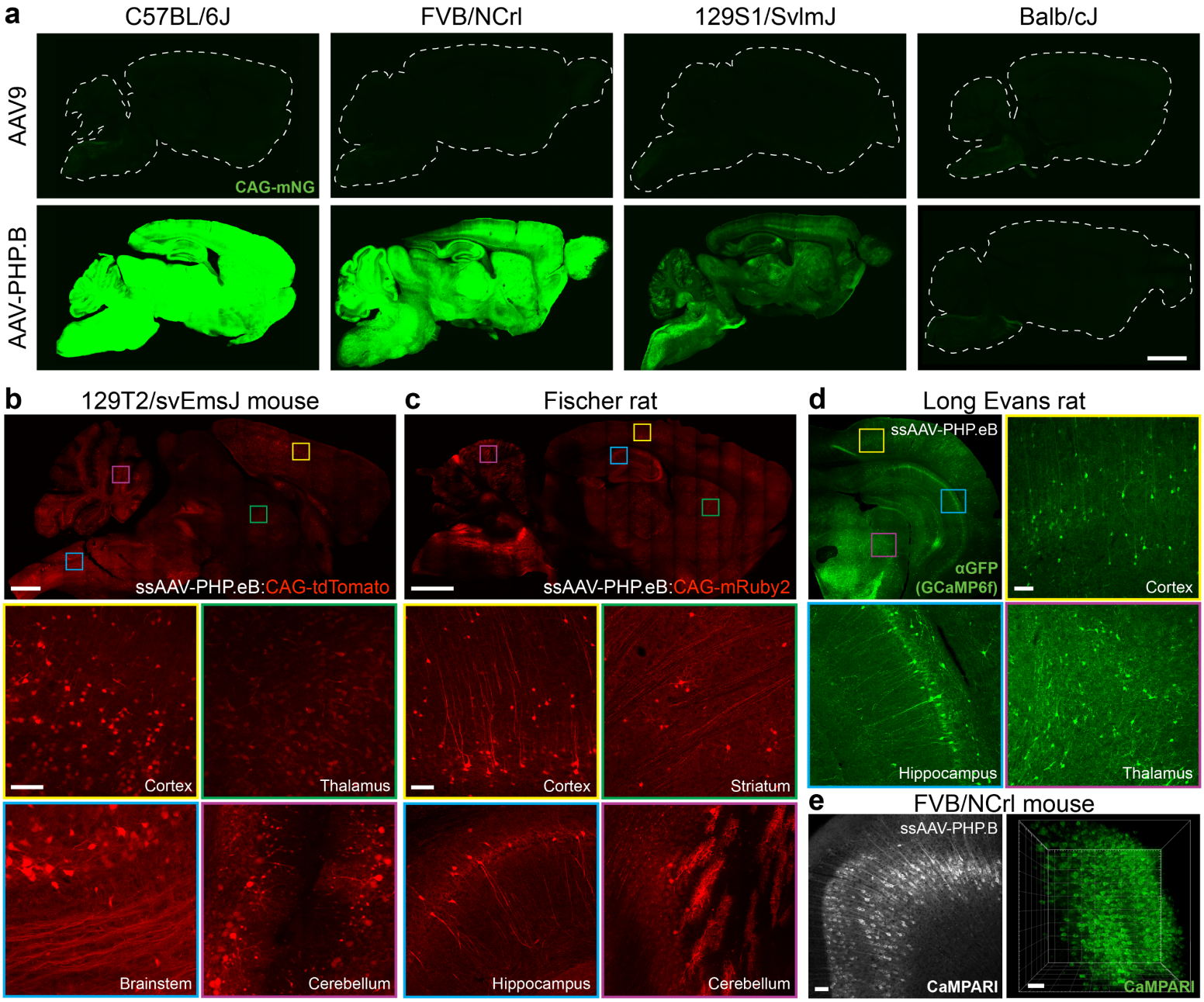
a–c, We used AAV-PHP.eB to package single-stranded (ss) rAAV genomes that express fluorescent reporters (XFPs), each with two nuclear localization signals (NLS), from cell type–specific promoters. Genomes containing the hSyn1, MBP, or GFAP ($GfABC_1D$) promoters were used to target neurons, oligodendrocytes, or astrocytes, respectively. Viruses were co-delivered by retro-orbital injection to 7-week-old C57BL/6N mice (n = 2) at 3×10^{11} vector genomes (vg)/virus (9×10^{11} vg total). Native fluorescence in coronal brain sections was evaluated 4 weeks later using confocal microscopy. All sections were mounted in Prolong Diamond Antifade before imaging. **a**, Cell type–specific, nuclear-localized XFPs label distinct cell types throughout the brain. Tile scan of a coronal brain slice, presented as a maximum-intensity projection; inset shows a zoomed-in view of the hippocampus. XFPs were mNeonGreen (mNG; green), tdTomato (tdT; red), and mTurquoise2 (mTurq2; blue). Scale bars, 1 mm and 500 µm (inset). **b,c**, Antibody staining can be used to determine the specificity and efficiency of cell type–specific promoters. **b**, Brain sections were stained with NeuN (purple), Olig2 (light blue), and S100 (purple) to mark neurons, oligodendrocyte lineage cells, and a population of glia that consists mainly of astrocytes, respectively. NLS-mNG (green), NLS-tdT (red), and NLS-mTurq2 (dark blue) indicate nuclear-localized XFPs. Images are from a single z plane. Scale bar, 100 µm. **c**, AAV-PHP.eB differentially transduces various regions and cell types throughout the brain. 'Specificity' or 'Efficiency' are defined as the ratio of double-labeled cells to the total number of XFP- or antibody-labeled cells, respectively. For image processing, median filtering and background subtraction using morphological opening were first applied to each image to reduce noise and correct imbalanced illumination. Each nucleus expressing XFPs and labeled with antibodies was then segmented by applying a Laplacian of Gaussian filter to the pre-processed images. We considered cells that were both expressing XFPs and labeled with antibodies if the nearest center-to-center distance between blobs (nuclei or cell bodies) in two channels was <7 µm (half of the cell body size). Five images per brain region were analyzed in each mouse; we excluded images with tissue edges because bright edges prevent accurate cell detection. Mean ± s.e.m. is shown. **d,e**, miRNA target sequences (TS) miR-204-5p or miR-708-5p[77] can be used to achieve expression that is more restricted to neurons or astrocytes, respectively. **d**, The indicated pairs of vectors were separately packaged into AAV-PHP.eB and co-administered via retro-orbital injection to 6- to 8-week-old C57BL/6J mice (n = 2) at 1×10^{11} vg/virus (2×10^{11} vg total); mNG and control XFP fluorescence were evaluated 3 weeks later using confocal microscopy. The CAG-mNG genome (green) contained no miRNA TS (left) or three tandem copies of miR-204 (middle) or miR-708 (right) TS; the CAG-XFP genome (magenta) contained no miRNA TS and was injected as an internal control. miR-204 reduced expression in cells with the morphology of astrocytes, and miR-708 reduced expression in cells with neuronal morphology. Scale bar, 100 µm. **e**, ssAAV-PHP.eB:CAG-GCaMP6f-3x-miR122-TS (left) or ssAAV-PHP.eB:CAG-GCaMP6f-3x-miR204-5p-3x-miR122-TS (right) was injected into 6- to 8-week-old C57BL/6J mice (n = 2) at 1×10^{11} vg/mouse; gene expression was evaluated 3 weeks later using confocal microscopy. The miR-204 TS reduced GCaMP6f expression (green) in S100+ glia (magenta) in the cortex. Both vectors contained three tandem copies of miR-122 to reduce expression in hepatocytes[78]. Insets and asterisks highlight representative images of S100+ glia. Scale bars, 50 µm and 10 µm (insets). Refer to Table 1 for details of rAAV genomes. Experiments on vertebrates conformed to all relevant governmental and institutional regulations and were approved by the Institutional Animal Care and Use Committee (IACUC) and the Office of Laboratory Animal Resources at the California Institute of Technology. In our primary publication[2], results were obtained using the C57BL/6J mouse line. pA, polyadenylation signal; W, WPRE.



We used AAV-PHP.S to package single-stranded (ss) rAAV genomes that express fluorescent reporters from either neuron-specific (e.g., hSyn1 and TH (tyrosine hydroxylase)) or ubiquitous promoters (e.g., CAG). Viruses were delivered by retro-orbital injection to 6- to 8-week-old C57BL/6J or Cre transgenic mice, and transgene expression was evaluated 2–3 weeks later. Whole-mount tissues were optically cleared using either ScaleSQ[64] (**a** (right), **c**, and **d**) or RIMS[57] (**b**) and imaged using wide-field or confocal microscopy; confocal images are presented as maximum-intensity projections. a, ssAAV-PHP.S:hSyn1-mNeonGreen and ssAAV-PHP.S:CAG-DIO-mRuby2 were co-injected into a TH-IRES-Cre mouse at 1×10^{12} vg/virus (2×10^{12} vg total). Native mNeonGreen (green) and mRuby2 (red) fluorescence were assessed 2 weeks later using wide-field (left) or confocal fluorescence microscopy (right). Images are from the second to sixth thoracic (T2–T6) (left) and eighth cervical to second thoracic (C8–T2) (right) paravertebral ganglia, which provide sympathetic innervation to thoracic organs, including the heart. Arrows denote mNeonGreen+ nerve fibers. Scale bars, 1 mm (left) and 500 µm (right). **b**, ssAAV-PHP.S:CAG-DIO-eYFP was injected into a TRPV1-IRES-Cre mouse at 1×10^{12} vg; gene expression in a nodose ganglion was evaluated 3 weeks later. Scale bar, 200 µm. **c**, A mixture of three separate viruses (ssAAV-PHP.S:CAG-DIO-XFPs) was injected into a TRPV1-IRES-Cre mouse at 1×10^{12} vg/virus (3×10^{12} vg total); gene expression in a dorsal root ganglion was evaluated 2 weeks later. XFPs were mTurquoise2 (blue), mNeonGreen (green), and mRuby2 (red). Scale bar, 200 µm. **d**, ssAAV-PHP.S:mTH-GFP and ssAAV-PHP.S:hSyn1-tdTomato-f (farnesylated) were co-injected into a C57BL/6J mouse at 5×10^{11} vg/virus (1×10^{12} vg total); gene expression in the duodenum was assessed 22 d later. The image stack includes both the myenteric and submucosal plexuses. Inset shows a zoomed-in view of ganglia containing TH+ cell bodies (green); tdTomato-f (red) labels both thick nerve bundles and individual fibers. Scale bars, 200 µm (left) and 50 µm (right). Refer to Table 1 for details of rAAV genomes. Experiments on vertebrates conformed to all relevant governmental and institutional regulations and were approved by the Institutional Animal Care and Use Committee (IACUC) and the Office of Laboratory Animal Resources at the California Institute of Technology. In our primary publication[2], results were obtained using the ChAT-IRES-Cre driver mouse line.



AAV-PHP.S viruses were delivered by retro-orbital injection to 6–8-week-old C57BL/6J or Cre transgenic mice. **a**, AAV-PHP.S transduces the heart more efficiently than the current standard, AAV9. ssAAV9:CAG-NLS-GFP or ssAAV-PHP.S:CAG-NLS-GFP were injected into C57BL/6J mice at 1×10^{12} vg/mouse. Native GFP fluorescence was assessed in whole-mount hearts 4 weeks later using wide-field fluorescence microscopy (unpaired t test, $t_7 = 8.449$, ****P <0.0001). For AAV9 and AAV-PHP.S, n = 5 and 4 mice, respectively. a.u., arbitrary units. Mean ± s.e.m. is shown. Scale bar, 3 mm. **b**, A mixture of three viruses (ssAAV-PHP.S:CAG-XFPs) was injected into a C57BL/6J mouse at 3.3×10^{11} vg/virus (1×10^{12} vg total); gene expression in cardiac muscle was evaluated 11 d later. Individual cardiomyocytes can be easily distinguished from one another. Scale bar, 200 µm. **c**, A mixture of three viruses (ssAAV-PHP.S:CAG-DIO-XFPs) was injected into a TRPV1-IRES-Cre mouse at 1×10^{12} vg/virus (3×10^{12} vg total); gene expression in cardiac nerves was evaluated 2 weeks later. Scale bar, 50 µm. **d**, ssAAV-PHP.S:Ef1α-DIO-ChR2-eYFP was injected into ChAT-IRES-Cre mice (n = 2) at 1×10^{12} vg; gene expression in a cardiac ganglion was evaluated 3 weeks later (left). Ex vivo intracellular recordings were performed after 5 weeks of expression. Differential interference contrast (DIC) image (middle) shows the optical fiber for light delivery and electrode for concurrent intracellular recordings; inset shows a higher-magnification image of a selected cell (asterisk). Cholinergic neurons generated action potentials in response to 473-nm light pulses (5 Hz, 20 ms) (right). Scale bars, 50 µm (left), 300 µm (middle), and 10 µm (inset). Whole-mount tissues in **b**, **c**, and **d** (left) were optically cleared using ScaleSQ[64] and imaged using confocal microscopy; confocal images are presented as maximum-intensity projections. XFPs in **b** and **c** were mTurquoise2 (blue), mNeonGreen (green), and mRuby2 (red). Refer to Table 1 for details of rAAV genomes. The pAAV-Ef1α-DIO-ChR2-eYFP plasmid was a gift from K. Deisseroth, Stanford University (Addgene, plasmid no. 20298). Experiments on vertebrates conformed to all relevant governmental and institutional regulations and were approved by the Institutional Animal Care and Use Committee (IACUC) and the Office of Laboratory Animal Resources at the California Institute of Technology. In our primary publication[2], results were obtained using the ChAT-IRES-Cre driver mouse line.



a, AAV-PHP.B transduces the brain more efficiently than AAV9 in C57BL/6J, FVB/NCrl, and 129S1/SvImJ mice, but not in BALB/cJ mice. ssAAV9:CAG-mNeonGreen or ssAAV-PHP.B:CAG-mNeonGreen were systemically delivered to 6- to 8-week-old C57BL/6J (n = 1–2 mice per group), FVB/NCrl (n = 2 mice per group), 129S1/SvImJ (n = 2 mice per group), and BALB/cJ mice (n = 2 mice per group) at 1×10^{12} vg/mouse. 3 weeks later, sagittal brain sections were mounted in Vectashield and imaged using confocal microscopy. Imaging and display parameters are matched across all panels. Scale bar, 2 mm. **b–e**, Examples of AAV-PHP.B- and AAV-PHP.eB-mediated brain transduction for fluorescent labeling (**b**,**c**) and calcium imaging (**d**,**e**) in different mouse and rat strains. Gene expression was evaluated using confocal microscopy. **b**, ssAAV-PHP.eB:CAG-tdTomato (Addgene) was delivered by retro-orbital injection to a 10-week-old 129T2/SvEmsJ mouse at 3×10^{11} vg; tdTomato fluorescence (red) was examined 2 weeks later. Scale bars, 1 mm (top) and 100 µm (insets). **c**, ssAAV-PHP.eB:CAG-mRuby2 was administered by tail-vein injection to a 6-week-old female Fischer rat at 3×10^{12} vg; 3 weeks later, brain slices were mounted in Prolong Diamond Antifade for imaging. Scale bars, 2 mm (top) and 100 µm (insets). **d**, ssAAV-PHP.eB:CMV-hSyn1-GCaMP6f-3x-miR122-TS was delivered by tail-vein injection to a 4-week-old female Long-Evans rat at 1×10^{13} vg; 3 weeks later, brain slices were stained with a GFP antibody (green) for imaging. Scale bars, 1 mm (top left) and 100 µm (insets). The vector contained three tandem copies of miRNA target sequence (TS) miR-122 (CAAACACCATTGTCACACTCCA) to reduce expression in hepatocytes[78]. Images in **d** courtesy of M. Fabiszak/W. Freiwald lab, Rockefeller University. e, ssAAV-PHP.B:CaMKIIa-CaMPARI (calcium-modulated photoactivatable ratiometric integrator[79]) was administered by retro-orbital injection to a 8-week-old FVB/NCrl mouse at 3×10^{11} vg and cortical expression was assessed 2 weeks later. Images are a 50-µm maximum-intensity projection of the cortex (left) and 500-µm-thick ScaleSQ[64]-cleared 3D volume (right). Scale bars, 100 µm. Experiments on vertebrates conformed to all relevant governmental and institutional regulations and were approved by the Institutional Animal Care and Use Committee (IACUC) and the Office of Laboratory Animal Resources at the California Institute of Technology. In our primary publication[2], results were obtained using the C57BL/6J mouse line. CaMKIIa, calcium/calmodulin-dependent protein kinase type IIa; CMV, cytomegalovirus early enhancer element.