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SUPPLEMENTARY INFORMATION

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Multiplexed peroxidase-based electron microscopy labeling enables simultaneous visualization of multiple cell types

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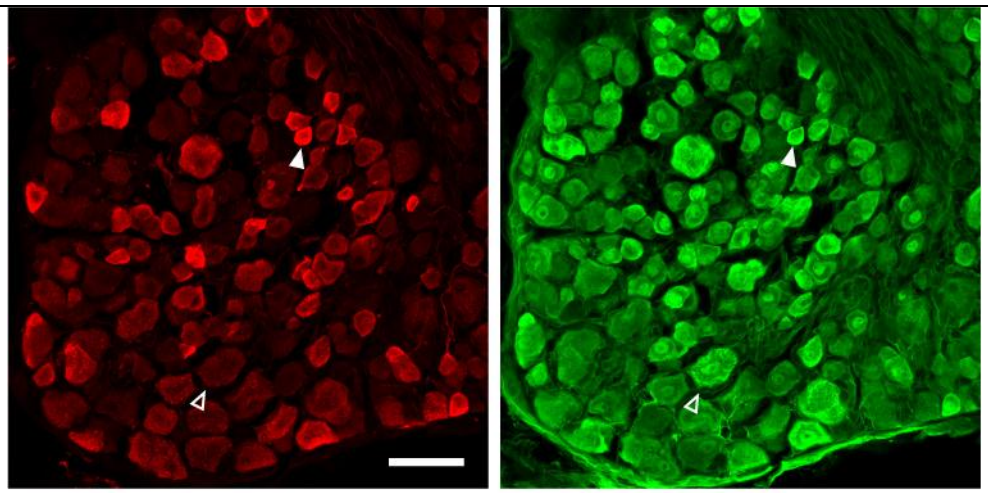
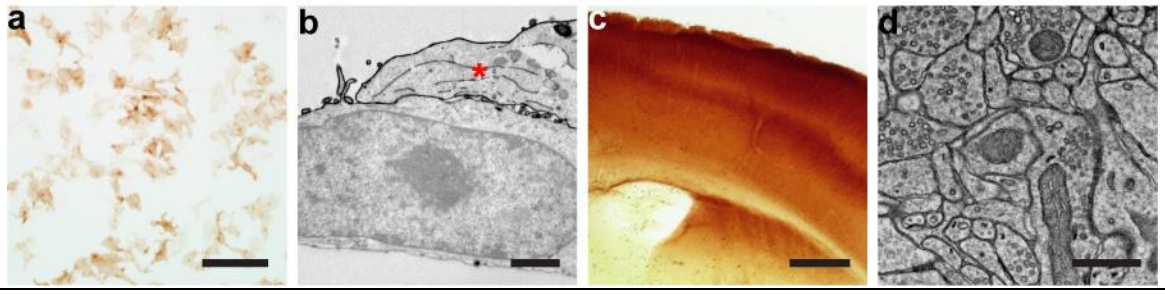


Supplementary Figure 1

AAV9 IP injection efficiently transduces DRG neurons

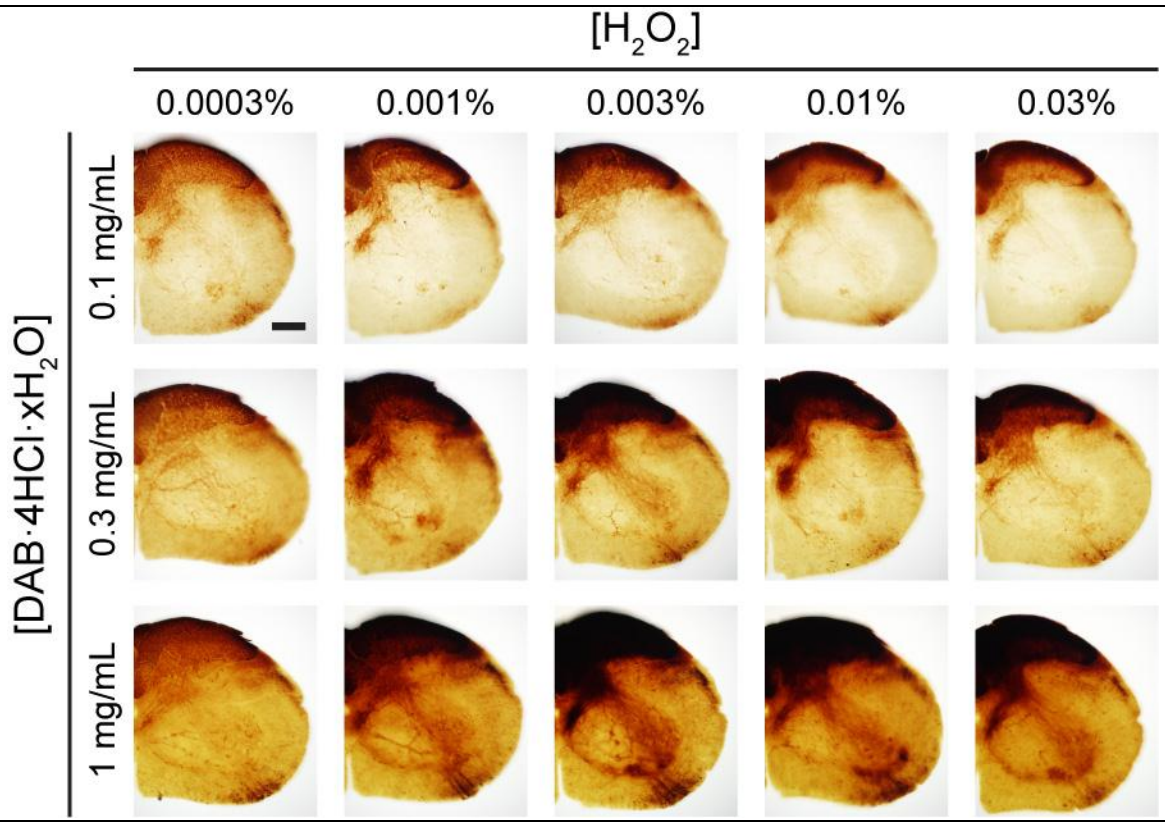
Confocal images showing the transduction efficiency of DRG neurons by neonatal AAV9 IP injections. AAV9-mCherry was used in this experiment. Note the efficient labeling but highly variable expression levels, with small-diameter neurons generally expressing the transgene at higher levels than large-diameter neurons. NeuN was used to label neurons. Solid arrowhead: example small-diameter neuron with high transgene expression level. Open arrowhead: example large-diameter neuron with low transgene expression level. n = 2 animals and experiments. Scale bar: 50 μm.



Supplementary Figure 2

Insufficient staining using previously reported constructs

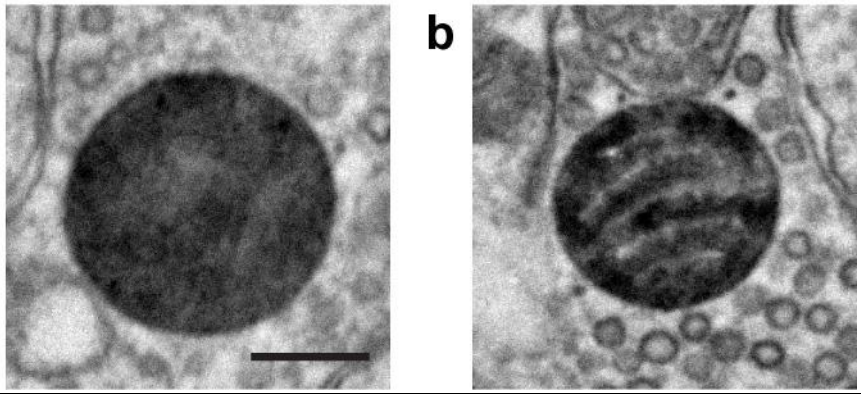
(**a**) LM image of HEK293T cells after transfection with HRP-TM. n = 3 experiments. (**b**) EM image showing plasma membrane labeling in a HEK293T cell transfected with HRP-TM (asterisk). n = 2 experiments. (**c**) LM image of the cortex after parenchymal injection of AAV1-HRP-TM. Staining could be observed with LM. n = 4 animals and experiments. (**d**) EM image of the cortex after parenchymal injection of AAV1-HRP-TM. Little if any discernible DAB staining was observed. n = 2 animals and experiments. Scale bars: **a**: 100 μm, **b**: 2 μm, **c**: 500 μm, **d**: 0.5 μm.



Supplementary Figure 3

Comparison of peroxidase staining conditions

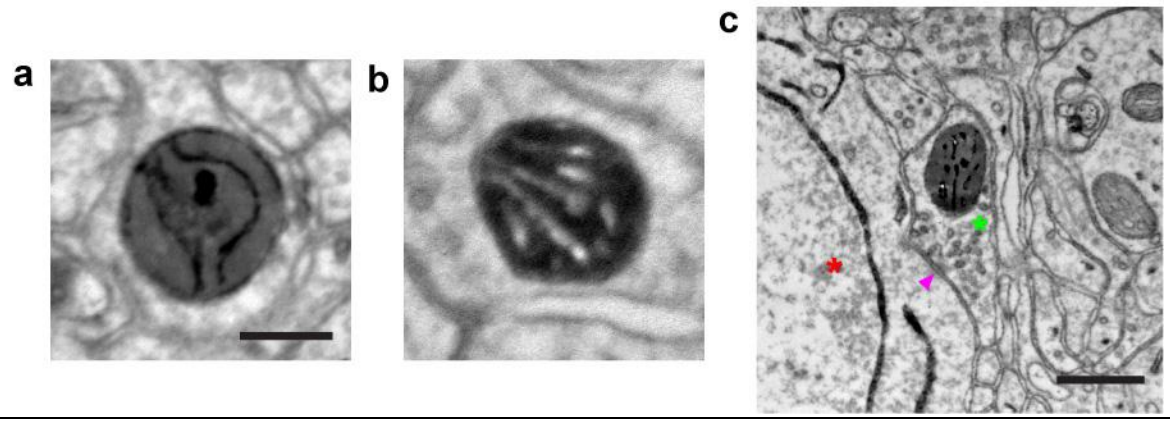
LM images of the spinal cord dorsal horn of the same animal after systemic transduction of AAV9-dAPEX2 stained with different conditions. 0.003% hydrogen peroxide gave the highest staining intensity regardless of the DAB concentration. Staining intensity observed under LM was positively correlated with DAB concentration. n = 2 animals and experiments. Scale bar: 200 μm.



Supplementary Figure 4

Excessively high concentrations of DAB could cause staining artifacts

(**a, b**) EM images of the spinal cord dorsal horn of the same animal after systemic transduction of AAV9-Matrix-dAPEX2 stained with 0.003% hydrogen peroxide and 1 mg/mL DAB (**a**) or 0.3 mg/mL DAB (**b**). The ultrastructure of the IMS was better preserved with 0.3 mg/mL DAB staining. n = 2 animals and experiments. Scale bar: 0.2 μm.



Supplementary Figure 5

Excessive osmication could cause staining artifacts

(**a, b**) EM images of the spinal cord dorsal horn of the same *Th*$^{T2A\text{-}CreER}$ animal transduced with AAV9-DIO-Matrix-dAPEX2 and treated with tamoxifen from P14-21 to label C-LTMRs prepared with the rOTO protocol (**a**) or the reduced osmium protocol (**b**). Strong spurious staining was seen in the IMS in the samples prepared with the rOTO protocol. This spurious staining was not seen in unlabeled mitochondria. Reduced osmium staining preserved the DAB staining while providing sufficient counterstaining contrast. n = 2 animals and experiments. (**c**) EM images showing a sample prepared with the rOTO protocol with double labeling of cortical layer 5 pyramidal neurons (ER) using Tg(Rbp4-Cre)KL100 and AAV1-DIO-ER-dAPEX2 (red asterisks), and fast-spiking GABAergic interneurons (mitochondrial matrix) using *Pvalb*$^{T2A\text{-}FlpO}$ and AAV1-FDIO-Matrix-dAPEX2 (green asterisks), equivalent to the experiment in **Fig. 3a**. Arrowhead: symmetric perisomatic synapse made by fast-spiking interneurons onto layer 5 pyramidal neurons. Note while spurious staining was present in the IMS, the mitochondrial matrix staining could still be easily distinguished from the ER staining. n = 2 animals and experiments. Scale bars: **a, b**: 0.2 μm, **c**: 0.5 μm.