

Supplementary Materials for  
**Expression of the excitatory opsin ChRER $\alpha$  can be traced longitudinally in  
rat and nonhuman primate brains with PET imaging**

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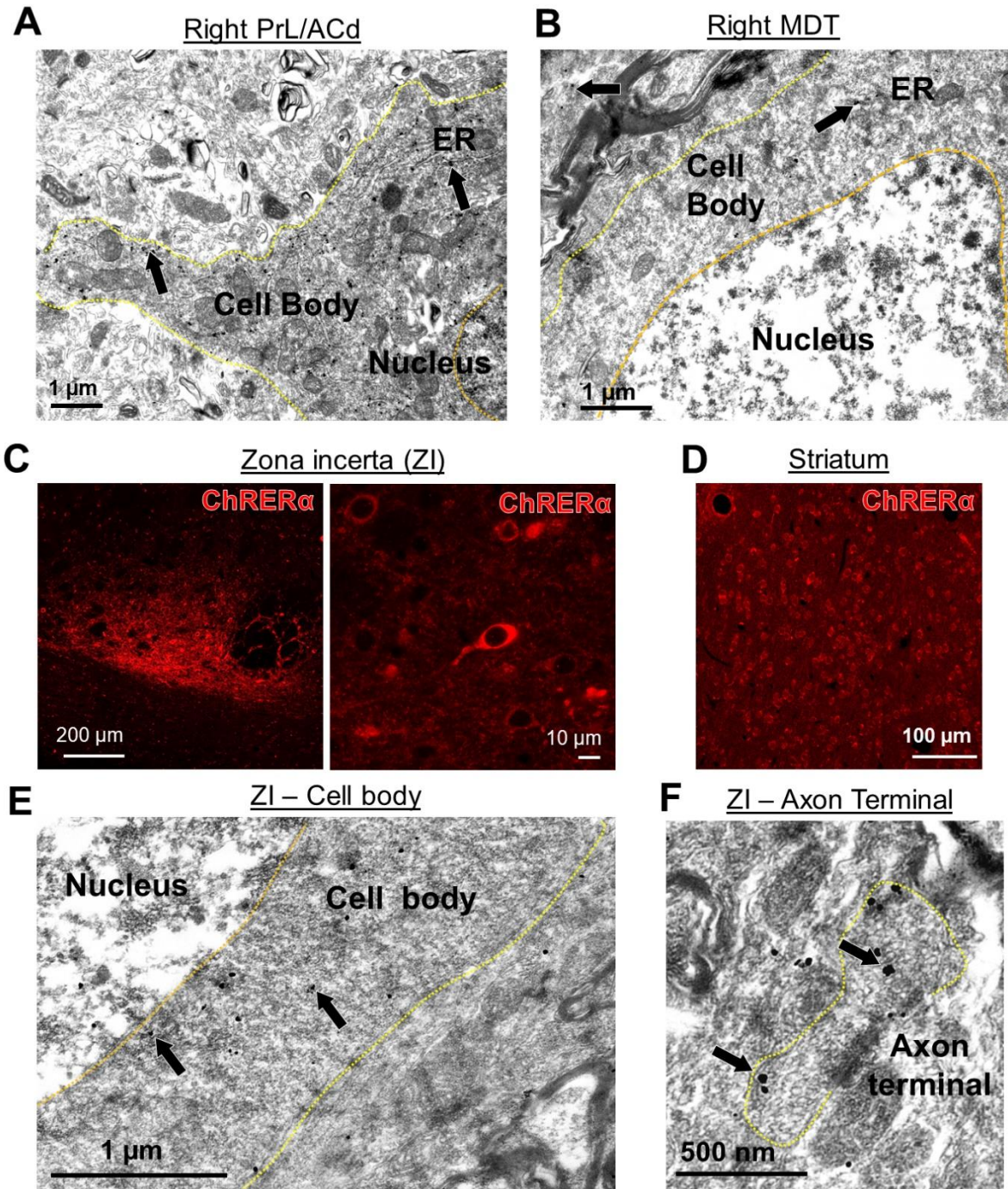
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**The PDF file includes:**

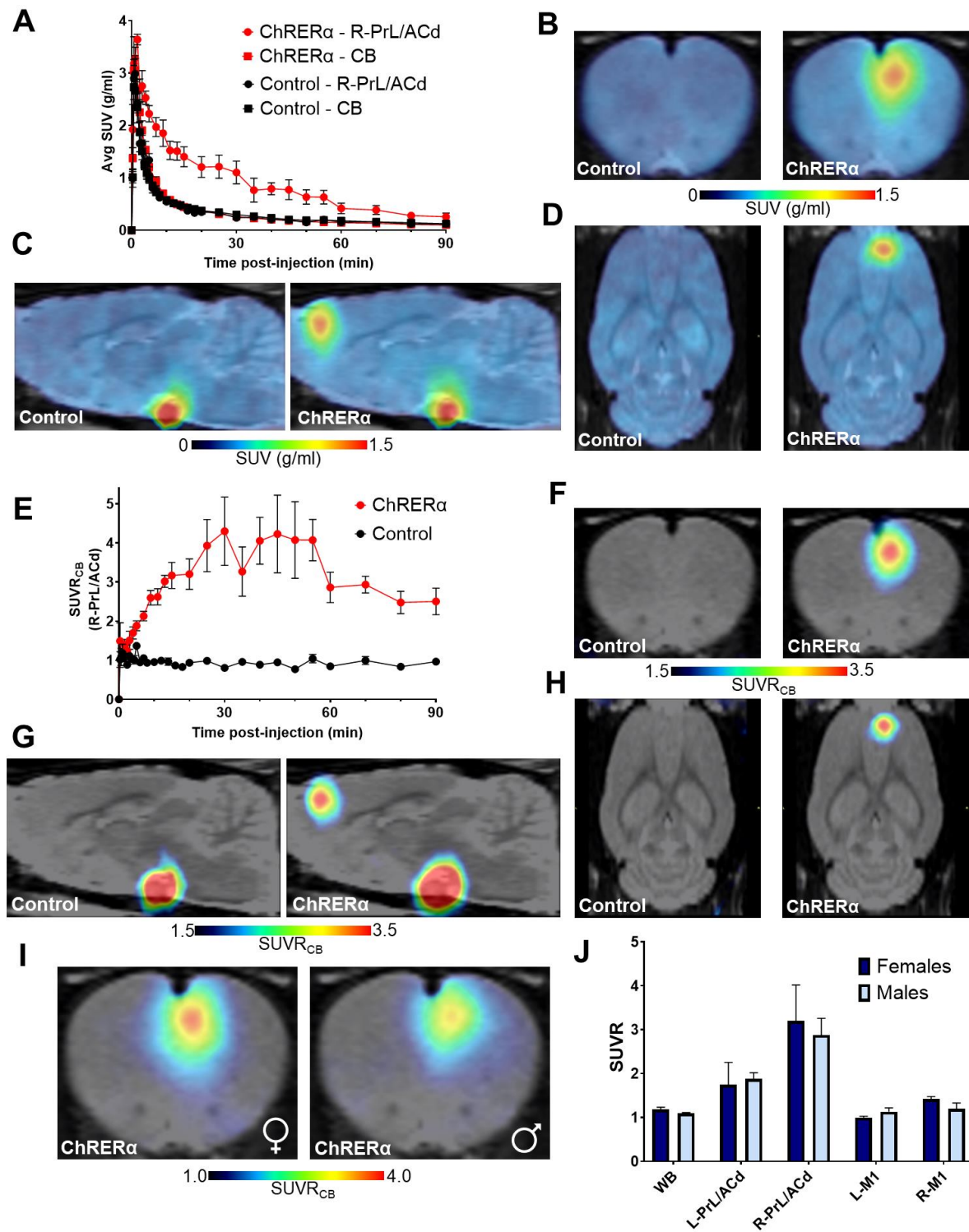
Figs. S1 to S9

**Other Supplementary Material for this manuscript includes the following:**

Data file S1  
MDAR Reproducibility Checklist

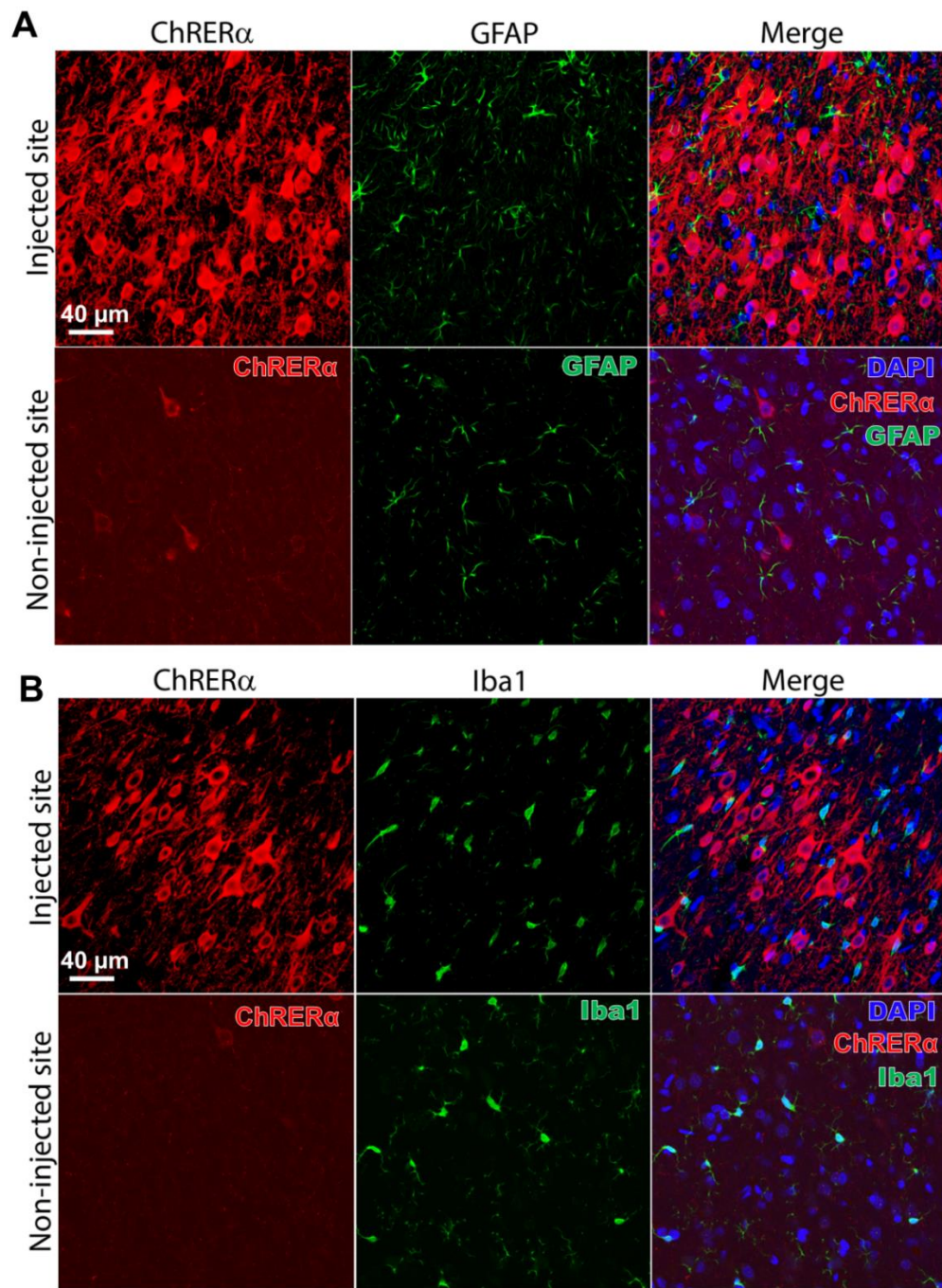


**Figure S1: Anti-ChR2 IHC and immuno-EM confirm ChRER $\alpha$  expression in the right PrL/ACd AAV site and in downstream projection areas in rats.** **A**, Immuno-EM image showing anti-ChR2 immunogold labeling of ChRER $\alpha$  in right PrL/ACd cells along the membrane and in the ER. **B**, Immuno-EM images in the MDT showing anti-ChR2 immunogold labeling of ChRER $\alpha$  in cells along the membrane and in the ER. **C**, IHC images showing anti-ChR2 labelling of ChRER $\alpha$  expressing cells in the right zona incerta (ZI). **D**, IHC images showing anti-ChR2 labelling of ChRER $\alpha$  expressing cells in the striatum. **E-F**, Immuno-EM images showing anti-ChR2 immunogold labeling of ChRER $\alpha$  in the cell body (**E**) and an axon terminal (**F**) of the right ZI.

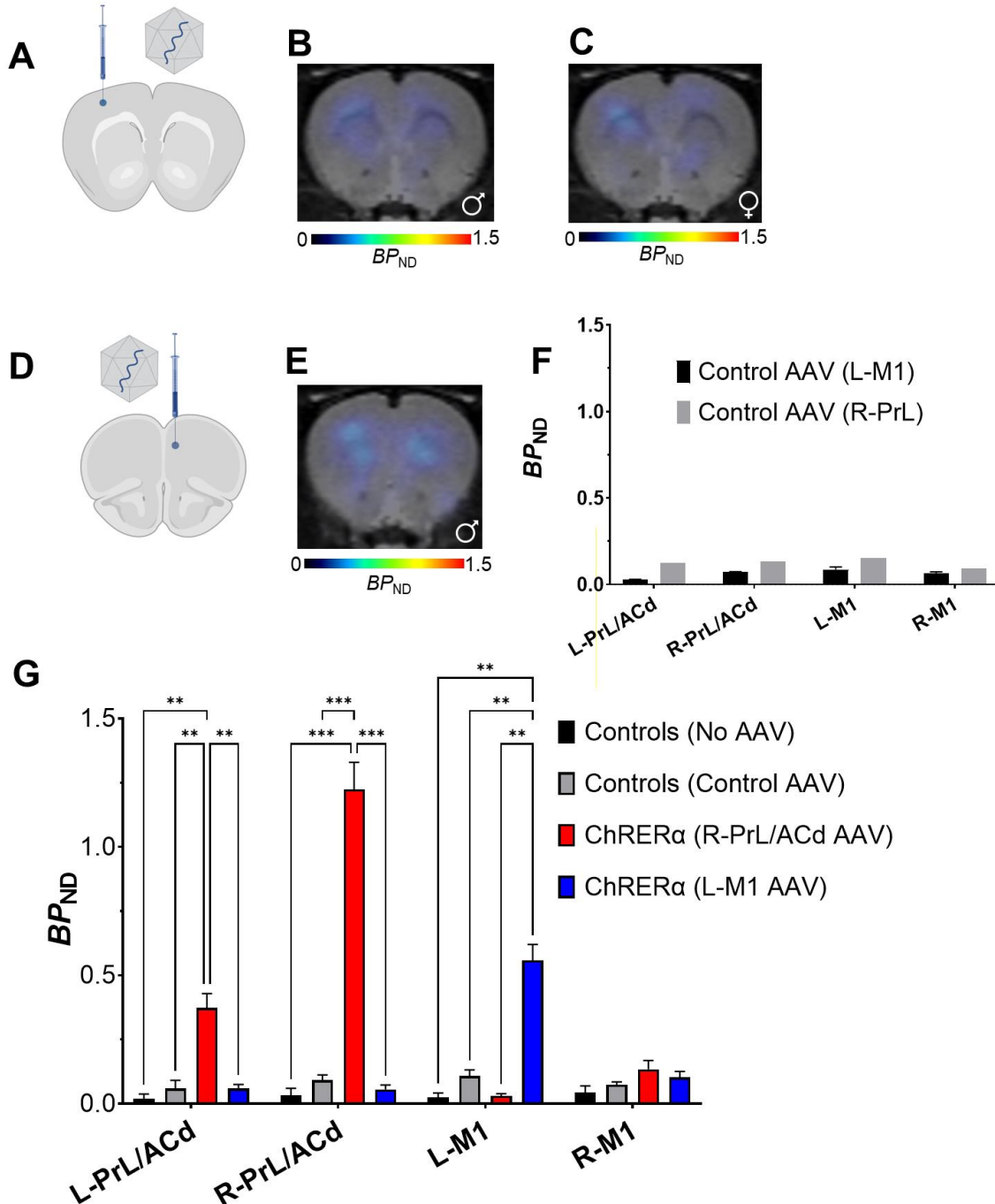


**Figure S2. Visualizing ChRER $\alpha$  in rats with  $[^{18}\text{F}]$ FES-PET (SUV and SUVR).** **A**, Time activity curves of average SUV (g/ml) in right PrL/ACd and cerebellum (CB) in control rats (black,  $n = 5$ ) and ChRER $\alpha$  rats 3-5 weeks post AAV (red,  $n = 6$ ). **B-D**, Average SUV (g/ml) 20-50min following injection with  $[^{18}\text{F}]$ FES. **E**, Time activity curves of average SUVR (CB ref) in control rats (black,  $n = 5$ ) ChRER $\alpha$  rats 3-5 weeks post AAV (red,  $n = 6$ ). **F-H**, Average SUVR (CB ref, 20-50min) in control ( $n=5$ ) and ChRER $\alpha$  rats ( $n=6$ ). **I-J**, Comparison of SUVR in female ( $n=3$ ) and male ( $n=3$ ) ChRER $\alpha$  rats.

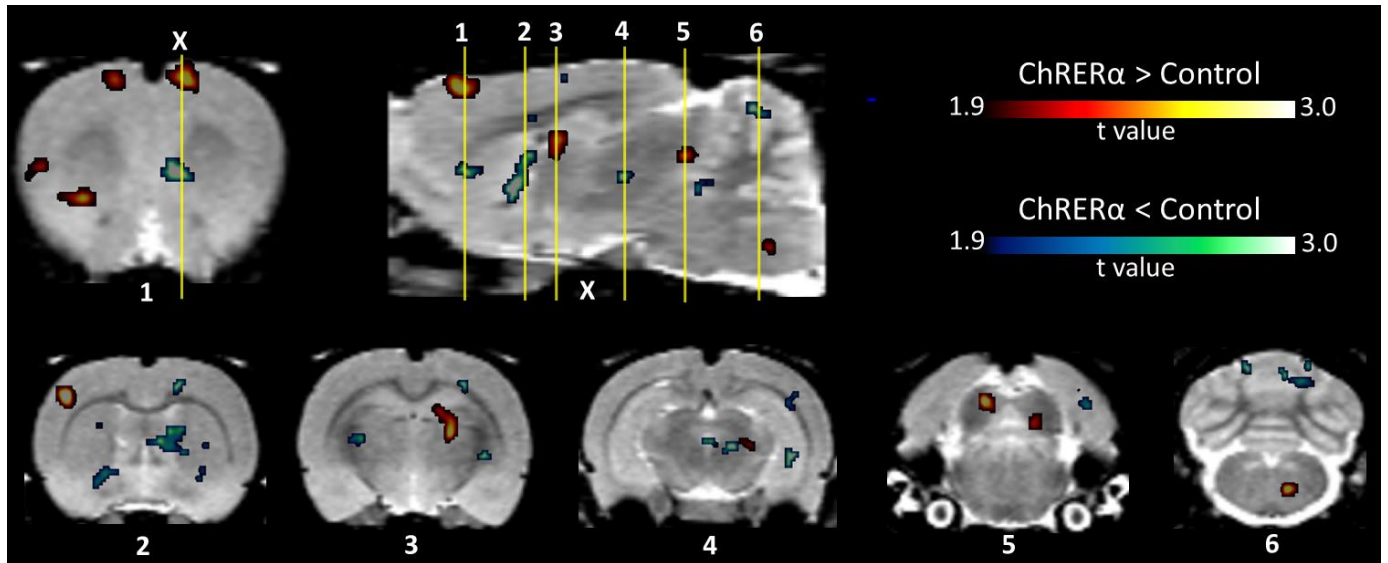




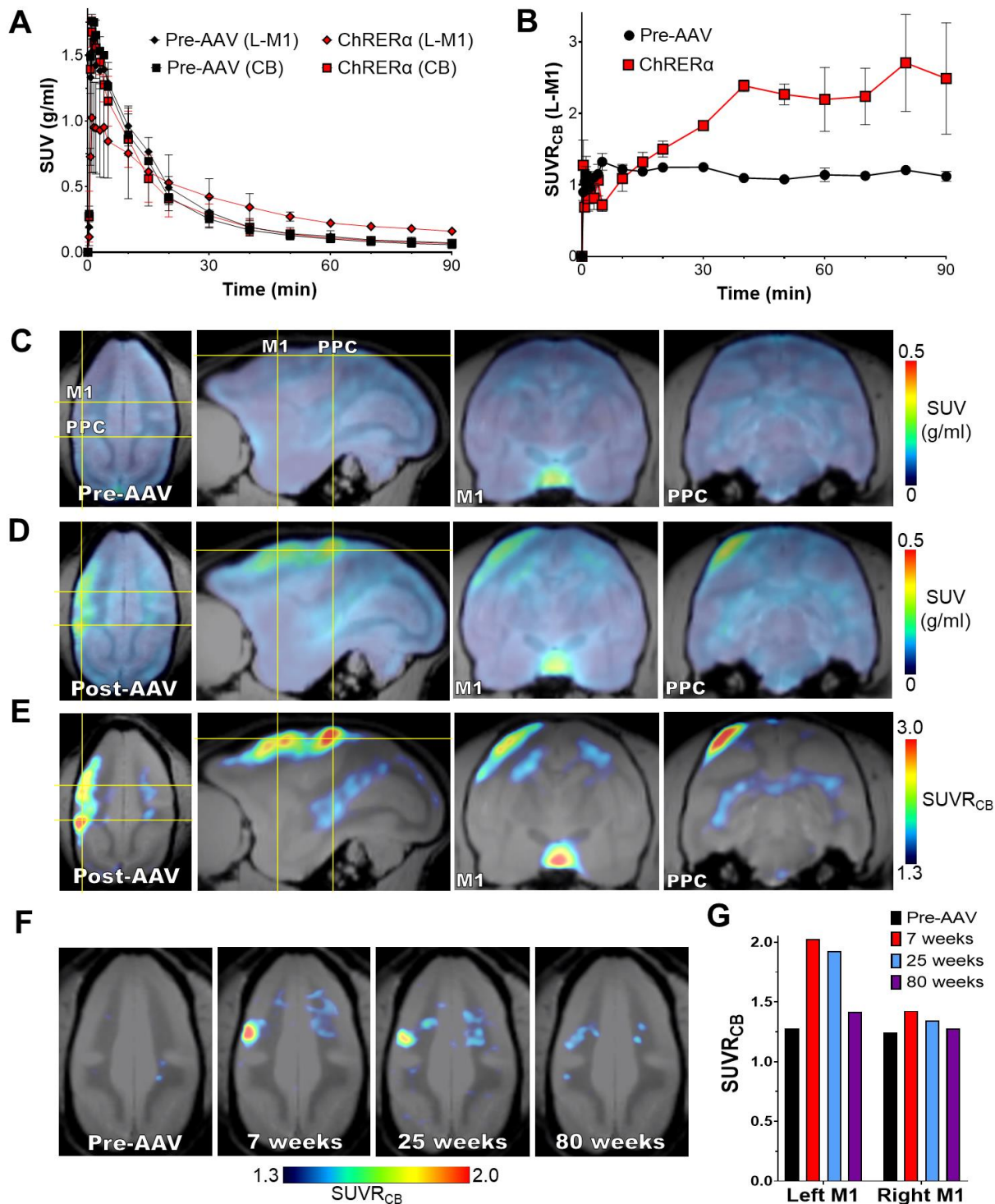
**Figure S3. Immunohistochemistry with antibodies for GFAP, Iba1 and ChR2 in coronal brain sections from rat injected with ChRER $\alpha$ -AAV in motor cortex shows no evidence of inflammatory response. A.** ChRER $\alpha$  (anti-ChR2) and GFAP (anti-GFAP) IHC in coronal brain section from rat injected with ChRER $\alpha$ -AAV in left M1. Representative images of GFAP staining in the AAV injected site (top images) and a non-injected site in contralateral M1 (bottom images), scale = 40  $\mu$ m. **B.** ChRER $\alpha$  (anti-ChR2) and Iba1 (anti-Iba1) IHC in coronal brain section from rat injected with ChRER $\alpha$ -AAV in left M1. Representative images of Iba1 staining in the AAV injected site (top images) and a non-injected site in contralateral M1 (bottom images), scale = 40  $\mu$ m.



**Figure S4. FES-PET in rats after injection with control AAV.** **A**, Left M1 injection site for control AAV. **B**, FES-PET scan in male rat injected with control AAV in left M1 (3 weeks post-AAV). **C**, FES-PET scan in female rat injected with control AAV in left M1 (3 weeks post-AAV). **D**, Right PrL/ACd injection site for control AAV. **E**, FES-PET scan in male rat injected with control AAV in right PrL/ACd (3 weeks post-AAV). **F**, VOI comparison of FES  $BP_{ND}$  in rats injected with control AAV in left M1 (black,  $n = 2$ ) or right PrL/ACd (grey,  $n = 1$ ). **G**, VOI comparison of FES  $BP_{ND}$  in control subjects with no AAV (black,  $n = 5$ ) or control AAV (grey,  $n = 3$ ), and ChRER $\alpha$  subjects with AAV in right PrL/ACd (red,  $n = 6$ ) or left M1 (blue,  $n = 5$ ); two-way ANOVA:  $F(9,45) = 62.86$ ,  $p < 0.0001$ ; Tukey's post hoc \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

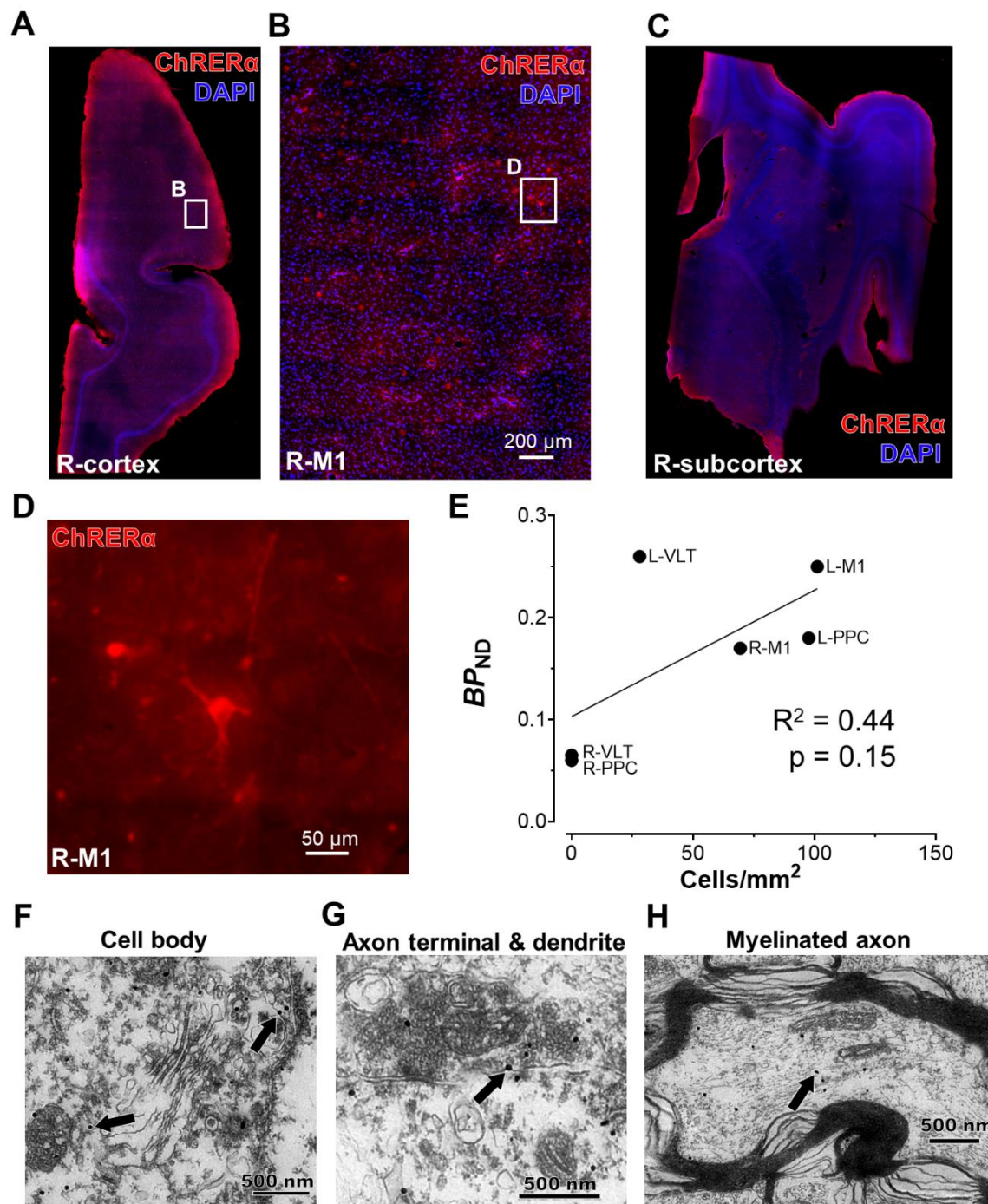


**Figure S5. Effects of light stimulation on brain activity in ChRER $\alpha$  rats (n = 5) vs. Control rats (n = 4) using FDG-PET.** Light stimulation in anesthetized ChRER $\alpha$  rats (3+ weeks after AAV injection in PrL/ACd) following bolus i.p. injection of FDG reveals brain areas with light-induced activation and inhibition in comparison to control rats (optic fiber in PrL/ACd but no AAV/opsin). Significant clusters with increased or decreased metabolic activity from a group voxel-wise analysis are shown below (ChRER $\alpha$  (n = 5) vs. Controls (n = 4); (df [1.0, 7.0],  $t = 1.89$ ,  $p < 0.05$ ;  $t = 3.0$ ,  $p < 0.01$ ). Numbers 1-6 label the coronal sections indicated by yellow lines shown in the sagittal slice (x).



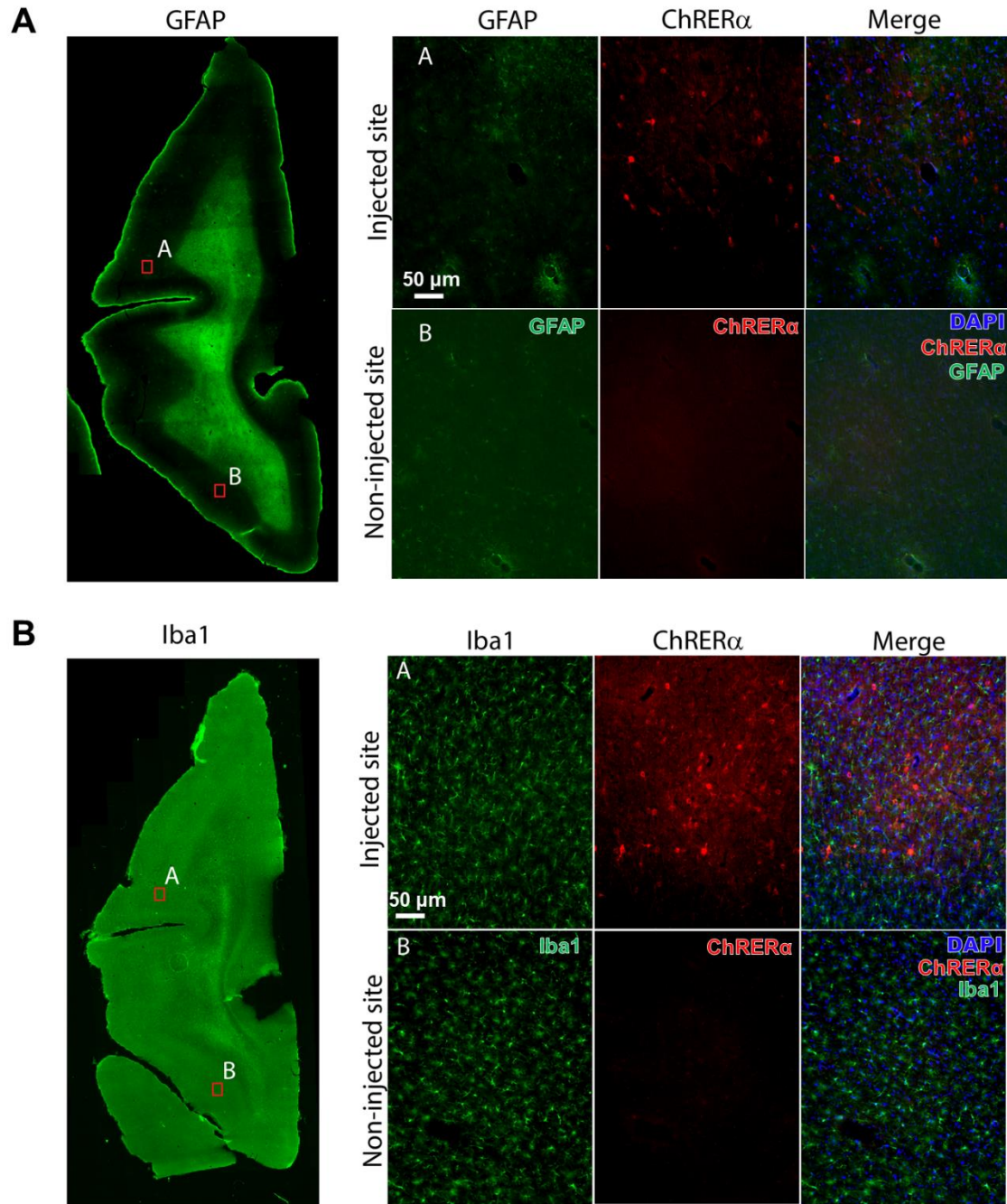
**Figure S6. Visualizing ChRERα in squirrel monkeys with  $[^{18}\text{F}]$ FES-PET.** **A**, Pre-AAV (black) and 5-7 weeks post AAV-ChRERα (red) time activity curves of average SUV (g/ml) in left M1 (L-M1) and cerebellum (CB) ( $n = 2$ ). **B**, Pre-AAV (black) and 5-7 weeks post AAV-ChRERα (red) time activity curves of average SUVR (CB ref) in left M1 (L-M1) ( $n = 2$ ). **C-D**, Average SUV (g/ml) 30-60min following injection with  $[^{18}\text{F}]$ FES in a squirrel monkey Pre-AAV (row **C**) and at 5 weeks post AAV-ChRERα (row **D**). **E**, Avg SUVR (CB ref) 30-60min following injection with  $[^{18}\text{F}]$ FES at 5 weeks post AAV-ChRERα. **F-G**, Longitudinal comparisons of avg SUVR (CB ref, 30-60min) in a second monkey.



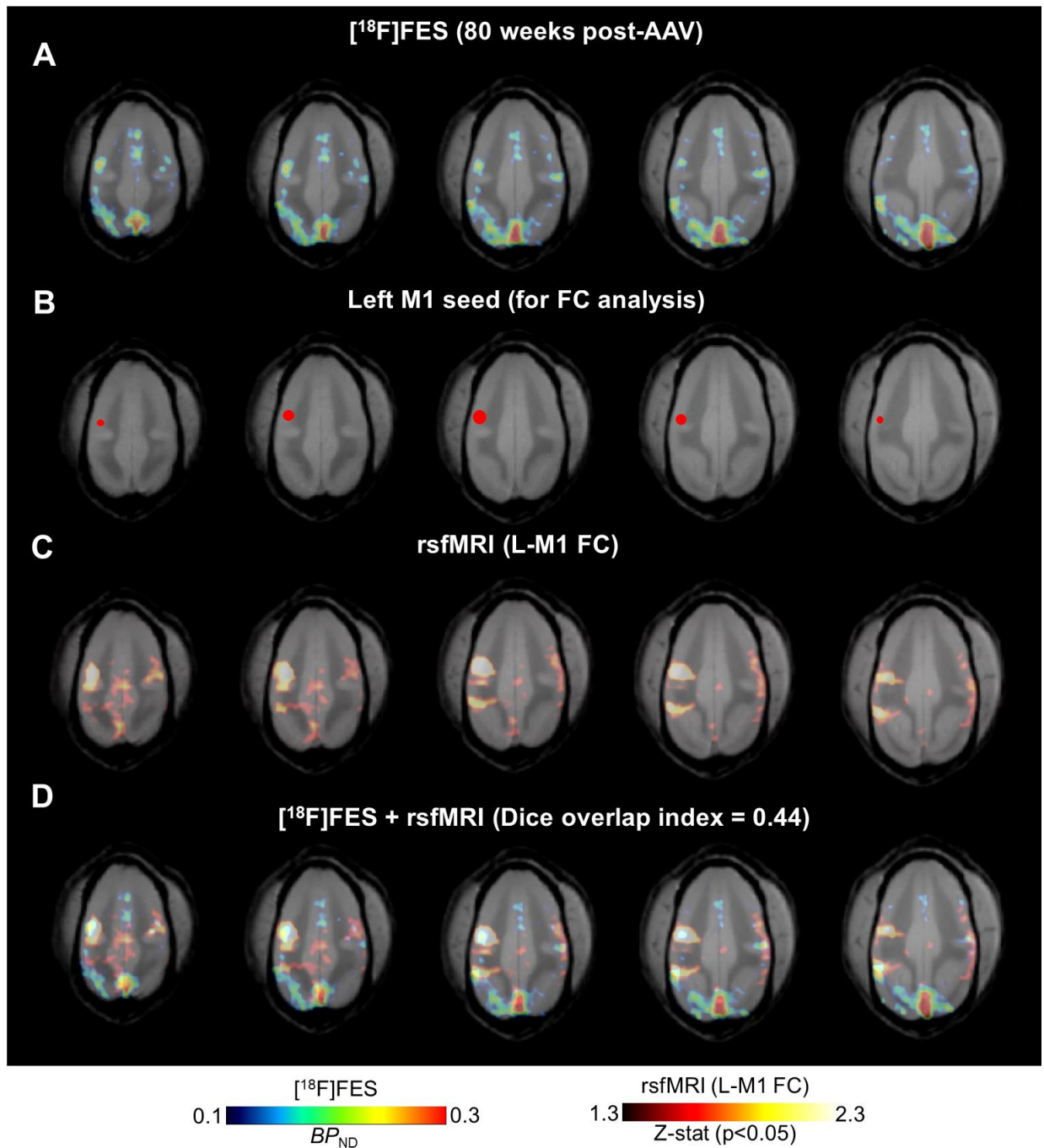


**Figure S7: Anti-ChR2 IHC and immuno-EM in squirrel monkey contralateral M1 and VLT.** A-B. IHC of a right cortical brain slice reveals ChR2 expression in right M1 (red = anti-ChR2, blue = DAPI), white rectangle in A indicates location of higher mag image shown in B, white rectangle in B indicates location of high mag image shown D. C. IHC of a right subcortical brain slice shows no ChR2 expression in right VLT. D. Anti-ChR2 labeling (red) confirms ChR2 expression in right M1. E. Correlation between regional FES-PET signal (i.e.,  $BP_{ND}$  at 80 weeks post-AAV) and anti-ChR2 IHC signal (positive cells per mm<sup>2</sup>) in monkey 2 ( $R^2 = 0.44$ ; two-tailed  $p = 0.015$ ,  $df = 4$ ). F-H, Anti-ChR2 immuno-EM reveals subcellular localization of ChR2 expression in right M1 (F) cell body, axonal terminal & dendrite (G), and myelinated axon (H).





**Figure S8. Immunohistochemistry with antibodies for GFAP, Iba1 and ChR2 in horizontal brain sections from squirrel monkey injected with ChRER $\alpha$ -AAV in left motor cortex shows no evidence of inflammatory response in tissue collected ~80 weeks post-AAV surgery. A.** ChRER $\alpha$  (anti-ChR2, red) and GFAP (anti-GFAP, green) IHC in horizontal brain section from squirrel monkey injected with ChRER $\alpha$ -AAV in left M1. On the left, low magnification image showing a left cortical section stained for GFAP. On the right, high magnification images of GFAP staining in the left M1 AAV injection site (top images) and a non-injected cortical site (bottom images), scale = 50  $\mu$ m. **B.** ChRER $\alpha$  (anti-ChR2, red) and Iba1 (anti-Iba1, green) IHC in horizontal brain section from squirrel monkey injected with ChRER $\alpha$ -AAV in left M1. On the left, low magnification image showing a left cortical section stained for Iba1. On the right, high magnification images of Iba1 staining in the left M1 AAV injection site (top images) and a non-injected cortical site (bottom images), scale = 50  $\mu$ m.



**Figure S9.  $[^{18}\text{F}]$ FES-PET and ChRER $\alpha$  predict functional brain connectivity in monkey 2.** **A.** Horizontal sections (left – most dorsal, right – most ventral) of  $[^{18}\text{F}]$ FES-PET (monkey 2) at 80 weeks post-AAV. **B.** Left M1 seed (red 2mm sphere centered at peak FES site from scan 1.5yr post-AAV) for functional connectivity analysis of resting state functional MRI (rsfMRI). **C.** rsfMRI functional connectivity patterns of the left M1 seed in an independent group of squirrel monkeys ( $n = 9$ , 35 total scans) co-registered to monkey 1 structural MRI. **D.** Overlapping patterns of  $[^{18}\text{F}]$ FES binding (ChRER $\alpha$  expression) and rsfMRI suggest structural and functional connectivity between left M1 and ipsilateral PPC, and in the contralateral hemisphere (Pearson correlation = 0.08;  $P=0$  with Fisher z-transformation, 29529 voxels, Dice overlap index = 0.44).