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Supplemental Information

Rationally Engineered AAV Capsids Improve Transduction and Volumetric Spread in the CNS

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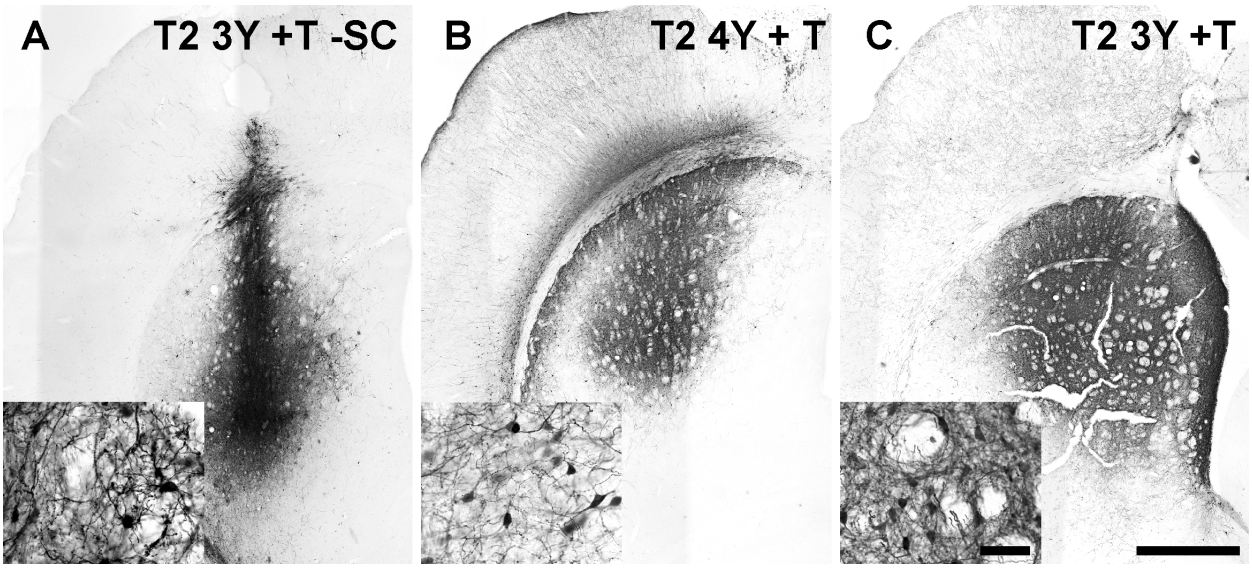

Figure S1. Incorporation of T-V mutations do not improve neuronal transduction. Adult Sprague-Dawley rats received intrastriatal injections of an AAV2 vector (2µl of 1.2 x 10^{12} vg/µl) as defined in **Table 1**. One month later the animals were sacrificed and processed for transgene (green fluorescent protein, GFP) immunoreactivity. **(a-c)** Representative images of GFP immunoreactivity in the striatum following the injection of (**a**) T2 3Y +T -SC (n = 8), (**b**) T2 4Y +T (n = 7), (**c**) T2 3Y +T (n = 8).

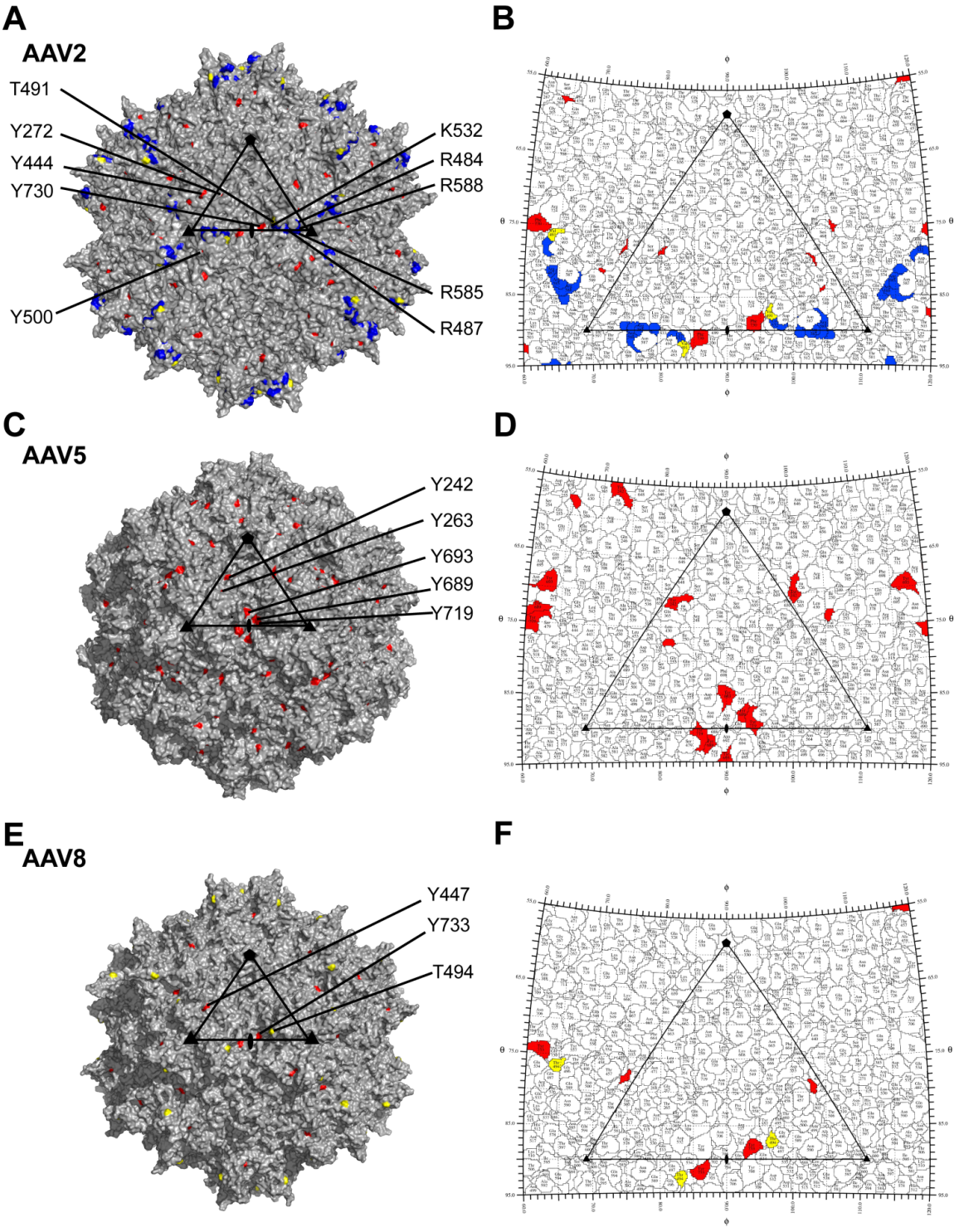

Figure S2. AAV capsid surface mutants. (**a,c,e**) Capsid surface densities for AAV2, AAV5, and AAV8, respectively, showing the positions of amino acids mutated (see **Table 1**). Tyrosine residues are in red, Threonine in yellow, and basic residues in blue. (**b,d,f**) "Roadmap" image showing the position of the mutated residues colored as in panels **a,c,e**. The triangle bounded by the two threefold axes (filled triangles) separated by the twofold axis (filled oval) and a fivefold (filled pentagon) depicts a viral asymmetric unit, 60 of which form the capsid.

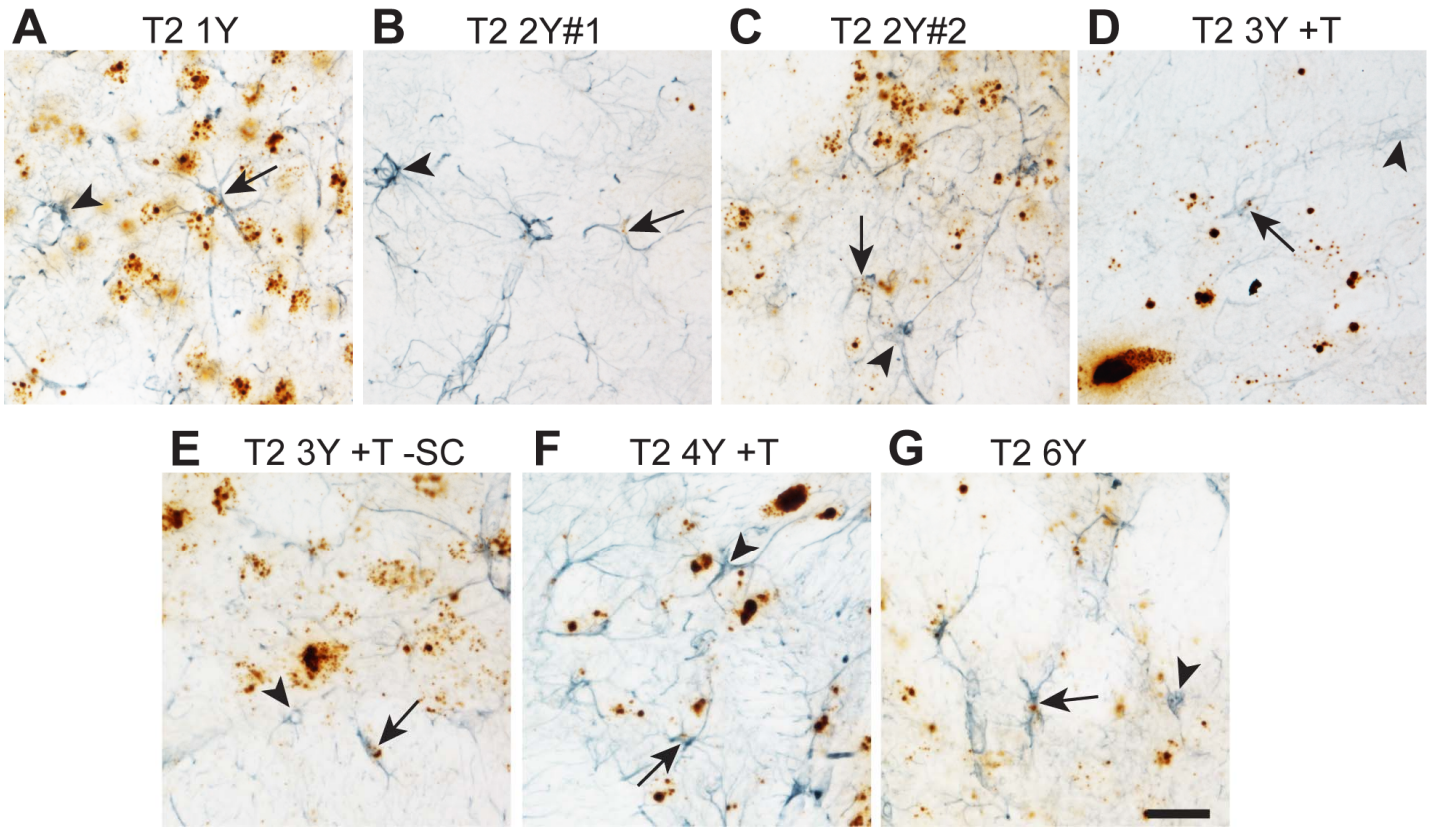

Figure S3. AAV2 capsid mutants display tropism for astrocytes. Adult Sprague-Dawley rats received intrastriatal injections of AAV vectors (2µl of 1.2 x 10^{12} vg/µl) as defined in **Table 1**. One month later, the animals were sacrificed and processed for dual label viral genome ISH (brown punctate staining) and astrocyte IHC (GFAP staining, blue/gray staining). **a-j** Representative sections from animals injected with T2 1Y (**a**), T5 2Y#1 (**b**), T2 2Y#2 (**c**), T2 3Y +T (**d**), T2 3Y +T -SC (**e**), T2 4Y +T (**f**), or T2 6Y (**g**). Brown ISH puncta were found within GFAP-labeled astrocytes with all of the AAVs tested (arrow heads). Scale bar in **g** is 25 µm and applies to all images.

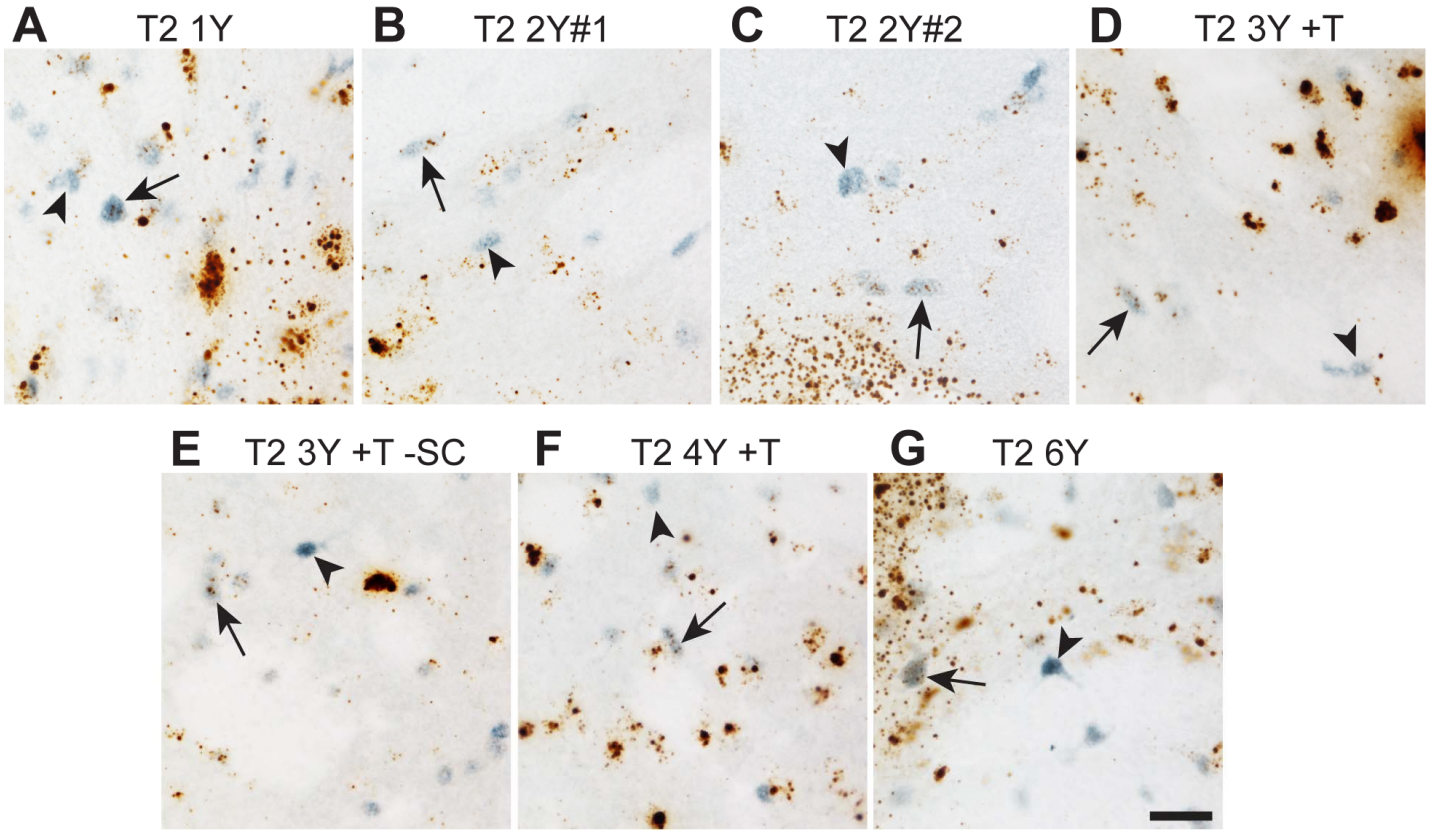

Figure S4. AAV2 capsid mutants display tropism for oligodendrocytes. Adult Sprague-Dawley rats received intrastriatal injections of AAV vectors (2µl of 1.2 x 10^{12} vg/µl) as defined in **Table 1**. One month later, the animals were sacrificed and processed for dual label viral genome ISH (brown punctate staining) and Oligo2 IHC (blue/gray staining). **a-j** Representative sections from animals injected with T2 1Y (**a**), T5 2Y#1 (**b**), T2 2Y#2 (**c**), T2 3Y +T (**d**), T2 3Y +T -SC (**e**), T2 4Y +T (**f**), or T2 6Y (**g**). Brown ISH puncta were found within Oligo2-labeled oligodendrocytes with all of the AAVs tested (arrow heads). Scale bar in **g** is 25 µm and applies to all images.

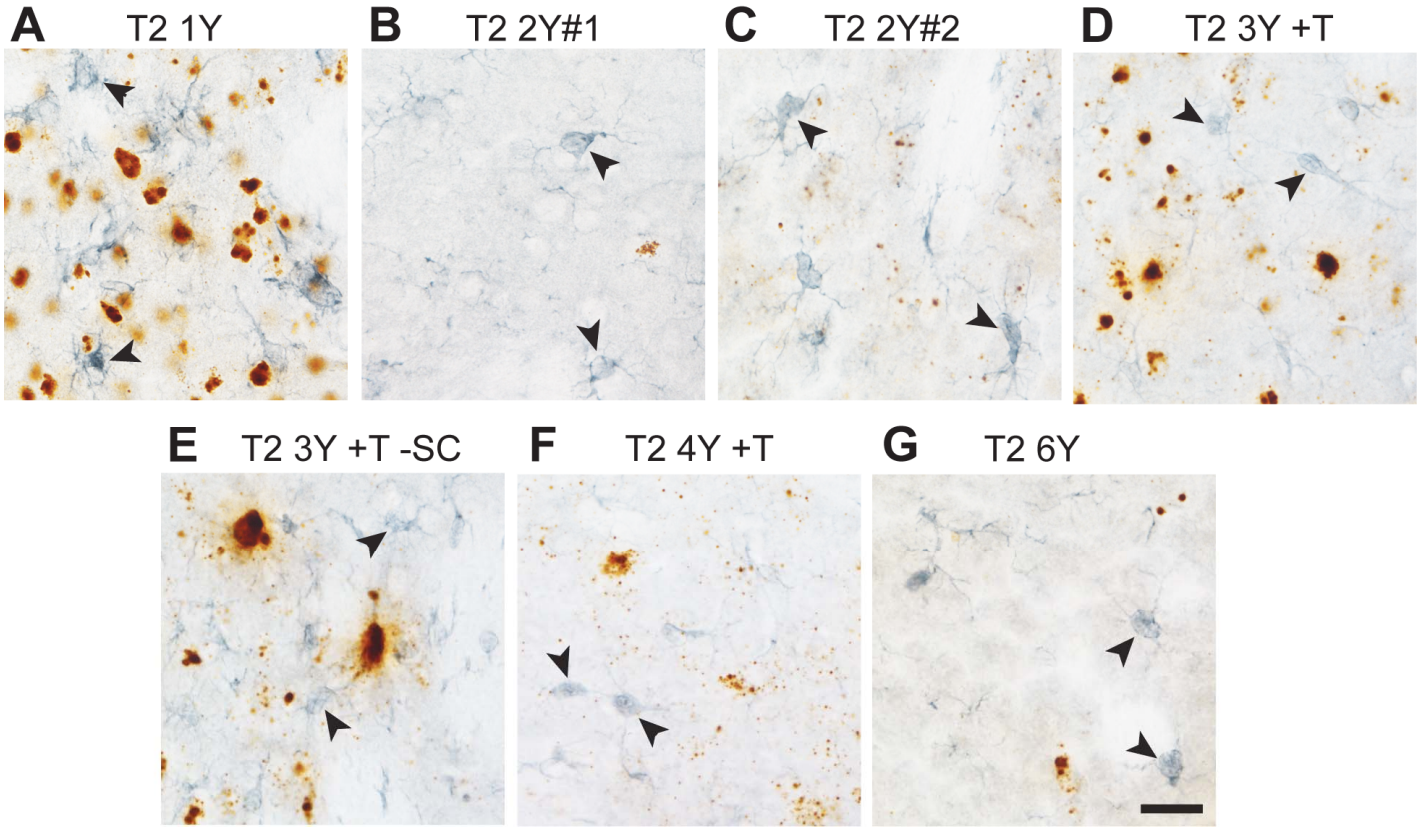

Figure S5. AAV2 capsid mutants do not confer microglial tropism. Adult Sprague-Dawley rats received intrastriatal injections of AAV vectors (2µl of 1.2 x 10^{12} vg/µl) as defined in **Table 1**. One month later, the animals were sacrificed and processed for dual label viral genome ISH (brown punctate staining) and microglia IHC (Iba1 staining, blue/gray staining). **a-j** Representative sections from animals injected with T2 1Y (**a**), T5 2Y#1 (**b**), T2 2Y#2 (**c**), T2 3Y +T (**d**), T2 3Y +T -SC (**e**), T2 4Y +T (**f**), or T2 6Y (**g**). Brown ISH puncta were not found within Iba1-labeled microglia with the AAVs tested (arrow heads). Scale bar in **g** is 25 µm and applies to all images.

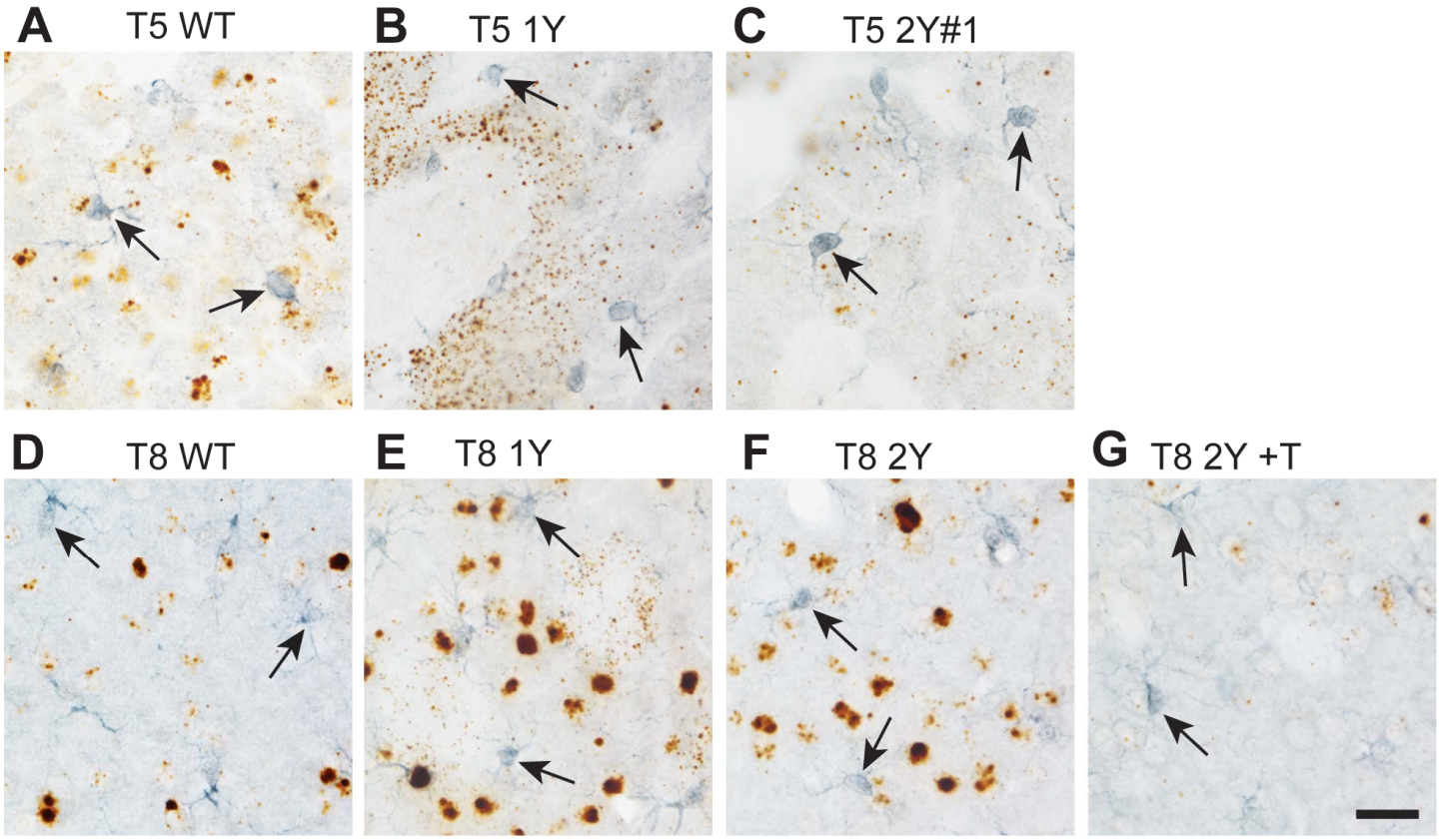

Figure S6. AAV5 and AAV8 capsid mutants do not confer microglial tropism. Adult Sprague-Dawley rats received intrastriatal injections of AAV vectors (2µl of 1.2 x 10^{12} vg/µl) as defined in **Table 1**. One month later, the animals were sacrificed and processed for dual label viral genome ISH (brown punctate staining) and microglia IHC (Iba1 staining, blue/gray staining). **a-j** Representative sections from animals injected with T5 WT (**a**), T5 1Y (**b**), T5 2Y#1 (**c**), T8 WT (**d**), T8 1Y (**e**), T8 2Y (**f**), or T8 3Y +T (**g**). Brown ISH puncta were not found within Iba1-labeled microglia with the AAVs tested (arrow heads). Scale bar in **g** is 25 µm and applies to all images.