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Virus Production and Administration

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Protocol for virus production and administration to mice in Yoo et al 2021

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Virus Production

1 Virus was produced using methods described in Challis et. al. (**Challis et al., 2019**)

2 Cells were grown in DMEM + Glutamax + Pyruvate (Gibco, Gaithersburg, MD-Stock# 10569-010) + 5% FBS + non-essential amino acids (Gibco, Gaithersburg, MD-Stock# 11140-050), penicillin-streptomycin (Gibco, Gaithersburg, MD-Stock# 15070-063).

3 Briefly, human embryonic kidney (HEK293T) cells were triple-transfected with pUCmini-iCAP-AAV-PHP.S, pHelper Plasmid, and one of the following pAAV genomes (CAG-NLS-GFP, hSYN1-tDTomato, GFAP-tDTomato, hSYN1-mRuby2, hSYN1-DiO-mRuby2, hSYN1-mNeonGreen, hSYN1-DiO-mNeonGreen, hSYN1-mTurquoise2, hSYN1-DiO-mTurquoise2, hSYN1-DiO-hM3Dq-mRuby2, CAG-GCaMP6F) in a DPBS + polyethylenimine (PEI)

4 Virus is precipitated from cells and supernatant with an 8% PEG solution (wt/vol), and purified by ultracentrifugation using 15%, 25%, 40%, 60% stacked iodixanol gradients.

Virus Administration

5 Virus was titered to 10^{12} viral genomes (vgs) and resuspended to a volume of 100µl with sterile PBS.

6 Mice were anesthetized using 2% isoflurane, and virus was injected retro-orbitally.

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