Supplemental Figure Legends

Figure S1, related to Figure 1. rAAV2-retro mediates efficient access to projection neurons in the rat. (A) Schematic of the example injection. Separate lots of rAAV2-retro expressing EGFP or tdTomato were injected in the striatum or superior colliculus, respectively, of an adult Long Evans rat. **(B–E)** Projection neurons in various brain regions labeled through these localized injections were imaged in coronal sections three weeks following virus delivery. Scale bars: 2 mm (B), 1 mm (E).

Figure S2, related to Figure 2. No naturally-occurring AAV serotypes match rAAV2-retro performance in corticopontine circuit. Representative images of corticopontine labeling in sagittal sections lateral to the injection tract (~1 mm lateral with respect to midline) taken from *Rosa26*-Lox-STOP-Lox-H2B-EGFP animals injected in the basal pons with various AAV serotypes carrying the Cre recombinase transgene. Scale bar: 1 mm.

Figure S3, related to Figure 2. Differences in retrograde efficiency between rAAV2-retro and naturally occurring AAV serotypes cannot be explained by differences in functional titers. (A) Schematic of the analysis. Sagittal sections taken from *Rosa26*-Lox-STOP-Lox-H2B-EGFP animals injected in the basal pons with rAAV2, rAAV9 and rAAV2-retro carrying Cre recombinase that span the middle of the injection site were identified by the presence of an injection tract (arrow). The midbrain region around the basal pontine nuclei (BPN) was then used for the analysis. **(B)** Representative images of pontine labeling for rAAV2, rAAV9 and rAAV2-retro. Scale bar: 1 mm. **(C)** Quantification of infection efficiency at the injection site (see Experimental Procedures).

Figure S4, related to Figure 1. Reduced heparin affinity for rAAV2-retro compared to its parental serotype AAV2. (A) Schematic of the heparin binding assay. 10^{11} purified genomic particles were loaded onto a heparin column previously equilibrated with 150 mM NaCl and 50 mM Tris at pH 7.5. Elution was then performed by increasing the concentration of NaCl in steps of 50 mM. **(B)** Fraction of virus eluted with increasing concentrations of NaCl. To quantify the amount of eluted virus, a small fraction of each elution was used to infect HEK293T cells, and the percentage of GFP positive cells was quantified 48

hours post-infection by flow cytometry. The load fraction represents the virus recovered in the column flow-through after sample loading in 150 mM NaCl. Error bars represent the S.D.