Supplementary Data

Supplementary Materials and Methods

AAV Production

293T cells were transfected with a plasmid carrying the viral genome to be packaged and a helper plasmid expressing the adenoviral helper genes, the AAV2 *Rep* genes, and the capsid genes of the respective serotype. To produce AAV9-GFP these plasmids were, respectively, pTRUF11 and pDG-9. For AAV1-LacZ these plasmids were pAAV-LacZ and pXYZ1. For AAV1-Serca2a these plasmids were pTR-Serca2a and pXYZ1. Transfections were done with linear PEI as a transfection reagent. For one triple-flask with an area of 500 cm^2, 50 μg of viral genome plasmid and 150 μg of helper plasmid were added, while vortexing, to 20 ml of serum-free DMEM followed by 700 μl of PEI (1 mg/ml, pH 4.5); incubated for 15 min at room temperature; and then added to the cells. Three days after transfection cells and supernatant were collected. For AAV9-GFP the purification was done as previously reported (Zeltner *et al.*, 2010) with the following exceptions: virus in the cell culture medium was precipitated by adding 31.3 g of ammonium sulfate per 100 ml of medium followed by incubation for 30 min on ice. The viral precipitate was collected by centrifugation at $8300 \times g$ for 30 min, using a JA-14 rotor in a Beckman Coulter Avanti J-E centrifuge. The pellet was resuspended in 9 ml of lysis buffer (150 m*M* sodium chloride, 50 m*M* Tris-HCl, pH 8.5). This solution was treated identically as the cell lysate. For AAV1-LacZ and AAV1-SERCA2a the purification was the same as for AAV9-GFP except that a second iodixanol gradient was performed by diluting the syringe-collected AAV iodixanol solution with 2 volumes of 1× TD (1× PBS, 1 m*M* magnesium chloride, 1.25 m*M* potassium chloride) buffer and underlaying this solution consecutively with 4 ml of 40% iodixanol and 4 ml of 60% iodixanol. AAV was collected by puncturing a hole in the bottom of the tube, collecting 1.25-ml fractions, and measuring viral genome concentrations by qPCR. The peak fractions (usually F4 and F5, F1 being the first fraction from the bottom of the tube) were pooled and dialyzed using Spectra/Por 2 12–14,000 MWCO tubing (Thermo Fisher Scientific, Pittsburgh, PA), with two overnight incubations with 100 volumes of lactate Ringer's solution.

Supplementary Table S1. Titers of Individual AAV Preparations

	AAV2-RSS (KC)	*AAV2-RSM (MSSM)*	*wt-AAV2a*	*wt-AAV2b*	*wt-AAV2c*	*AAV9-GFP*	*AAV1-LacZ*	*AAV1-SERCA*
Exp. 1	1.06×10^9	9.42×10^8	2.97×10^9	7.50×10^9	6.90×10^9	2.71×10^9	3.68×10^9	2.63×10^9
Exp. 2	7.56×10^8	7.32×10^8	3.21×10^9	4.80×10^9	4.20×10^9	3.62×10^9	3.48×10^9	2.27×10^9
Exp. 3	1.70×10^9	8.05×10^8	2.26×10^9	4.25×10^9	5.32×10^9	4.39×10^9	3.90×10^9	2.55×10^9
Exp. 4	7.13×10^8	1.32×10^9	2.43×10^9	4.80×10^9	4.74×10^9	3.11×10^9		
Exp. 5	1.19×10^9							

AAV, adeno-associated virus; AAV2-RSS, AAV2 reference standard stock; GFP, green fluorescent protein; KC, viral titers determined at King's College London School of Medicine; LacZ, β-galactosidase; MSSM, viral titers determined at Mount Sinai School of Medicine; SERCA, sarco/endoplasmic reticulum calcium ATPase; wt, wild-type.

Note: The titers of each virus preparation were determined several times. The titers obtained in each individual experiment are listed as viral particles per microliter.