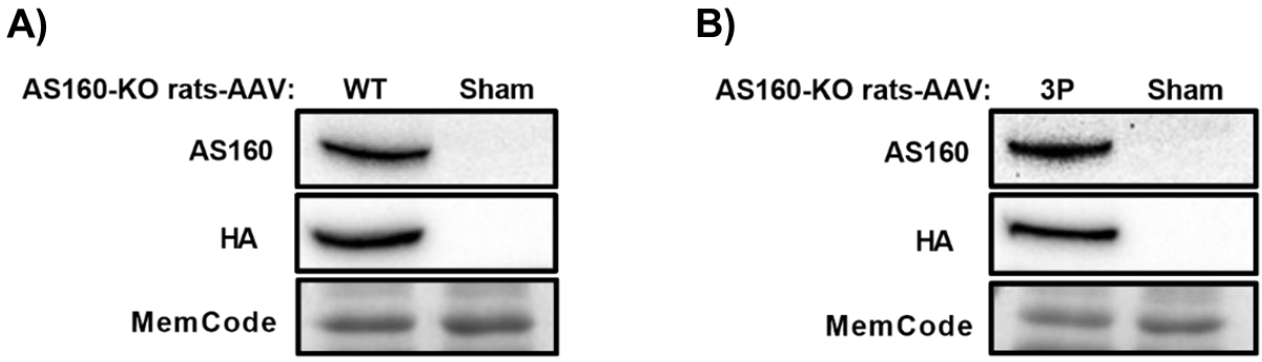
ONLINE SUPPLEMENTAL MATERIALS

AS160 Expression by AAV-AS160 Injected, but not Contralateral Epitrochlearis Muscle

To confirm that AAV-delivery of an AS160 construct to one epitrochlearis in an AS160-KO rat results in AS160 expression in that muscle, but not in the contralateral epitrochlearis, AS160 KO rats were injected with either AAV-WT-AS160 or AAV-3P-AS160 in one epitrochlearis, and the contralateral muscle underwent sham-treatment (Sham; identical surgical procedure as used for AAV-WT-AS160 and AAV-3P-AS160, except muscles were injected with vehicle, sterile PBS). Post-injection (3-4 weeks), muscles isolated from deeply anesthetized rats were processed and subjected to immunoblotting using anti-AS160 or anti-HA-tag (AS160 constructs included an N-terminal HA-tag). Two gels were loaded with a molecular weight standard in lane 1, and adjacent lanes were loaded with lysate prepared using a muscle (from AS160-KO rat) that was injected with AAV-WT-AS160, and the contralateral muscle (from the same rat) after sham-treatment. Another two gels were loaded similarly, except a lysate prepared using a muscle (from AS160-KO rat) that was injected with AAV-3P-AS160, and the contralateral muscle (from the same rat) after sham-treatment. MemCode was used as a loading control for each gel. For each AS160 construct, one immunoblot used anti-AS160 as the primary antibody, and the other immunoblot used anti-HA-tag as the primary antibody.

Muscles injected with either AAV-WT-AS160 (Suppl. Fig. 1A) or AAV-3P-AS160 (Suppl. Fig. 1B) were characterized by robust AS160 expression as assessed with either anti-AS160 or anti-HA-tag. AS160 was undetectable for either anti-AS160 or anti-HA-tag in lysates from AS160-KO rats that underwent sham-treatment (Sham). The MemCode loading control demonstrated similar total protein loading between the paired muscles. AS160 was undetectable in muscles that were not injected with either AAV-WT-AS160 or AAV-3P-AS160.

Supplemental Figure 1



Transduction Efficiency

Epitrochlearis muscles from AS160-KO rats were injected with AAV-WT-AS160, AAV-3P-AS160, or underwent sham-treatment (identical surgical procedure as used for AAV-WT-AS160 and AAV-3P-AS160, except muscles were injected with vehicle, sterile PBS). Subsequently (3-4 weeks later), muscles dissected from deeply anesthetized rats were incubated (at 35^{o}C while oxygenated with 95% O_2/5% CO_2, with continuous shaking) with Krebs-Henseleit Buffer (KHB) supplemented with 0.1% bovine serum albumin (BSA), 2 mM sodium pyruvate, and 6 mM mannitol for 30 min. Muscles were washed 3 times with 5 ml of Ca^{2+}-free KHB supplemented with 0.1% BSA and 8 mM glucose on ice. Subsequently, muscles were incubated for 60 min in Ca^{2+}-free KHB supplemented with 0.1% BSA, 8 mM glucose, and 3% collagenase Type 2. Under a dissecting microscope, in a petri dish containing KHB with 0.1% BSA, single fibers were gently isolated from the muscle fiber bundles using forceps. After isolation, each fiber was transferred by pipette with 10 µl of media to a microfuge tube. 15 µl of lysis buffer (T-PER supplemented with 1 mM Na_3VO_4, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate tetrabasic decahydrate, 1 mM β-glycerophosphate, 1 µg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride) and 25 µl of 2 × Laemmli buffer were added to the tube.

The tubes were then vortexed and heated to 95°C for 10 min. Resultant lysate was subjected to SDS-PAGE followed by immunoblotting with anti-HA-tag (AS160 constructs included an N-terminal HA-tag) or anti-Actin (loading control). In addition to lysates from single fibers, each gel was loaded with a molecular weight standard, a negative control (Neg Cnt; pooled single fibers from AS160-KO rat that underwent sham-treatment), and a positive control (Pos Cnt; muscle from AS160-KO rat that had been injected with AAV-WT-AS160).

Transduction efficiency was calculated for each muscle injected with an AAV-AS160 construct as follows: (number of fibers expressing HA-tag ÷ total number of fibers evaluated by immunoblotting) x 100%. Fibers were isolated from 4 muscles injected with AAV-AS160-WT (35-72 fibers/muscle; 179 total fibers; transduction efficiency = 96.1 ±3.2%). Fibers were isolated from 3 muscles injected with AAV-AS160-3P (35-95 fibers/muscle; 202 total fibers; transduction efficiency = 95.9 ±1.4%). Representative blots are provided (Suppl. Fig, 2A and 2B). Transduction efficiency was not significantly different for AAV-AS160-WT versus AAV-AS160-3P based on comparison using a t-test (Suppl. Fig. 2C).

Supplemental Figure 2