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Supplemental Information

Systemic AAV9.BVES delivery ameliorates muscular dystrophy in a mouse model of LGMDR25

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Supplementary Information

Table S1. ECG parameters of WT and BVES-KO male mice without or with neonatal injection of AAV9.BVES before and after exercise

Parameters	Before exercise			After exercise		
	WT	KO	neonatal (AAV9)	WT	KO	neonatal (AAV9)
RR (ms)	120.1±8.0	150.8±20.1*	140.7±25.0	107.8±9.3	133.6±26.1*	110.3±11.4#
PR (ms)	44.0±12.8	39.9±8.1	47.9±7.3	41.7±12.4	37.7±6.1	44.9±6.4
QRS duration (ms)	14.0±1.9	13.8±2.1	15.0±2.7	13.7±1.8	14.2±2.0	13.1±2.0
QT (ms)	29.2±11.5	36.6±8.0	37.3±6.6	30.4±10.9	35.9±9.7	37.6±10.1
QTc (ms)	84.5±33.4	97.3±19.5	102.1±16.0	92.7±34.8	94.3±36.1	115.6±32.3
QR (ms)	8.4±0.9	7.8±1.6	7.9±1.2	8.3±0.9	7.9±1.0	6.8±1.7

* indicate significant difference between WT and KO, # indicate significant difference between KO and AAV9.BVES

Table S2. ECG parameters of WT and BVES-KO male mice without or with adult injection of AAV9.BVES before and after exercise

Parameters	Before exercise			After exercise		
	WT	KO	adult (AAV9)	WT	KO	adult (AAV9)
RR (ms)	117.7±14.6	141.8±28.0	142.0±36.9	104.3±7.3	118.3±14.9*	102.4±5.3#
PR (ms)	47.0±10.4	47.0±8.9	44.8±10.8	39.6±13.1	40.9±13.9	45.7±21.5
QRS duration (ms)	13.3±1.9	13.6±2.1	13.5±3.1	12.6±1.4	12.7±2.6	14.8±2.6
QT (ms)	28.2±4.7	35.0±6.8	29.2±12.4	31.7±8.3	36.7±6.4	41.8±6.9
QTc (ms)	76.0±23.7	97.2±20.8	82.6±41.0	98.6±26.5	107.9±16.6	133.2±21.4
QR (ms)	7.4±1.4	7.8±1.4	6.7±1.6	6.8±1.4	6..2±1.4	7.4±1.9

* indicate significant difference between WT and KO, # indicate significant difference between KO and AAV9.BVES

Table S3. ECG parameters of WT and BVES-KO female mice without or with neonatal injection of AAV9.BVES before and after exercise

Parameters	Before exercise			After exercise		
	WT	KO	neonatal (AAV9)	WT	KO	neonatal (AAV9)
RR (ms)	119.9±8.8	154.0±50.7	124.5±13.0	113.8±6.8	123.0±7.4*	110.6±6.3#
PR (ms)	48.3±12.7	45.7±14.4	50.4±17.9	46.4±6.5	42.4±12.8	47.2±18.4
QRS duration (ms)	14.0±1.1	14.2±1.9	13.6±1.3	13.0±1.2	13.7±1.4	14.6±1.7
QT (ms)	31.8±9.1	38.0±8.3	40.9±10.5	37.8±7.7	35.1±8.1	35.6±15.2
QTc (ms)	92.8±27.6	96.7±18.4	116.9±31.0	112.3±23.6	101.0±22.7	106.4±46.1
QR (ms)	8.4±0.6	8.1±1.3	8.0±1.4	7.5±1.5	7.3±1.3	7.8±1.5

* indicate significant difference between WT and KO, # indicate significant difference between KO and AAV9.BVES

Table S4. Skipped heart beats of WT and BVES-KO male mice without or with AAV9.BVES

Mice with skipped heart beats/total mice	WT	KO	Neonatal (AAV9)	Adult (AAV9)
Before Exercise	0/17	10/21	2/10	0/7
After Exercise	1/17	6/21	1/10	0/7

Table S5. ECHO parameters of WT and BVES-KO male mice without or with neonatal injection of AAV9.BVES

Parameters	WT	KO	Neonatal (AAV9)
CO	19.9±3.9	17.1±4.7	18.4±3.5
LVEDD	3.9±0.3	3.8±0.5	4.1±0.4
LVESD	3.2±0.6	2.5±0.5	2.9±0.4
FS	34.4±3.3	34.6±6.3	32.7±7.2
LV Mass	138.8±42.1	128.9±32.5	145.9±31.3
LV Mass Corr	135.2±39.2	103.1±26.0	123.1±30.2
SV	42.4±6.9	38.7±7.9	42.2±7.7
EDV	66.5±11.6	56.5±12.9	74.7±16.7
ESV	24.1±6.1	23.5±11.0	32.5±10.3

Note: CO, cardiac output; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; FS, fractional shortening; LV mass, left ventricular mass; LV mass Corr, corrected left ventricular mass; SV, stroke volume; EDV, end-diastolic volume; ESV, end-systolic volume.

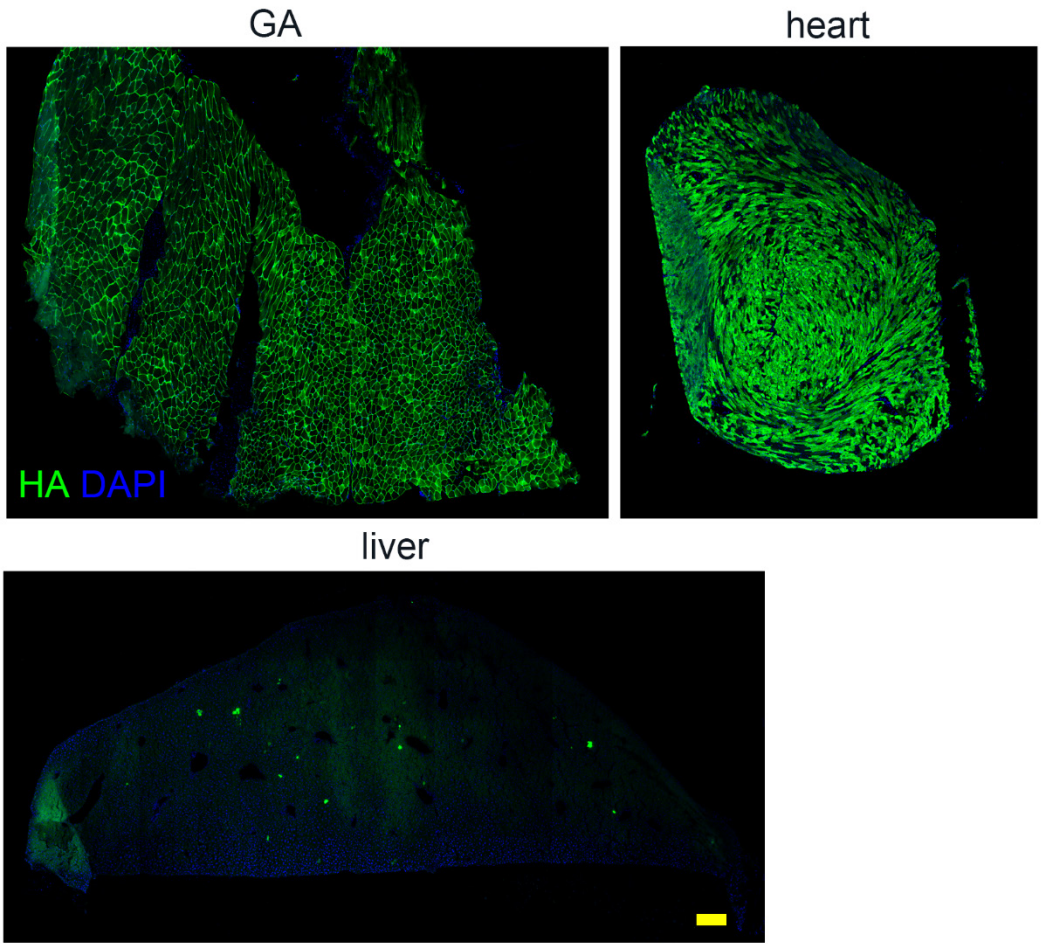

Figure S1. Immunofluorescence staining images showing the expression of BVES transgene in male BVES-KO mouse tissues. Representative, stitched immunofluorescence images of the entire heart, GA and liver sections stained with the anti-HA antibody from a 3-month-old BVES-KO mouse treated with AAV9.BVES at P3. Scale bars: 200 μm.

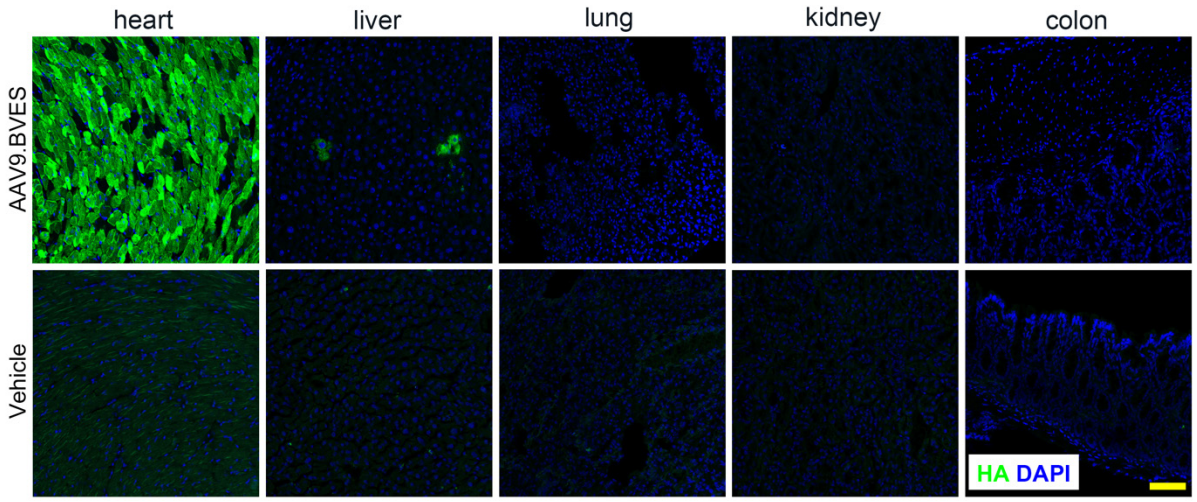

Figure. S2. Representative immunofluorescence staining images of various tissue sections from male BVES-KO mice following AAV9.BVES administration through IP injection at P3. Scale bar: 100 μm.

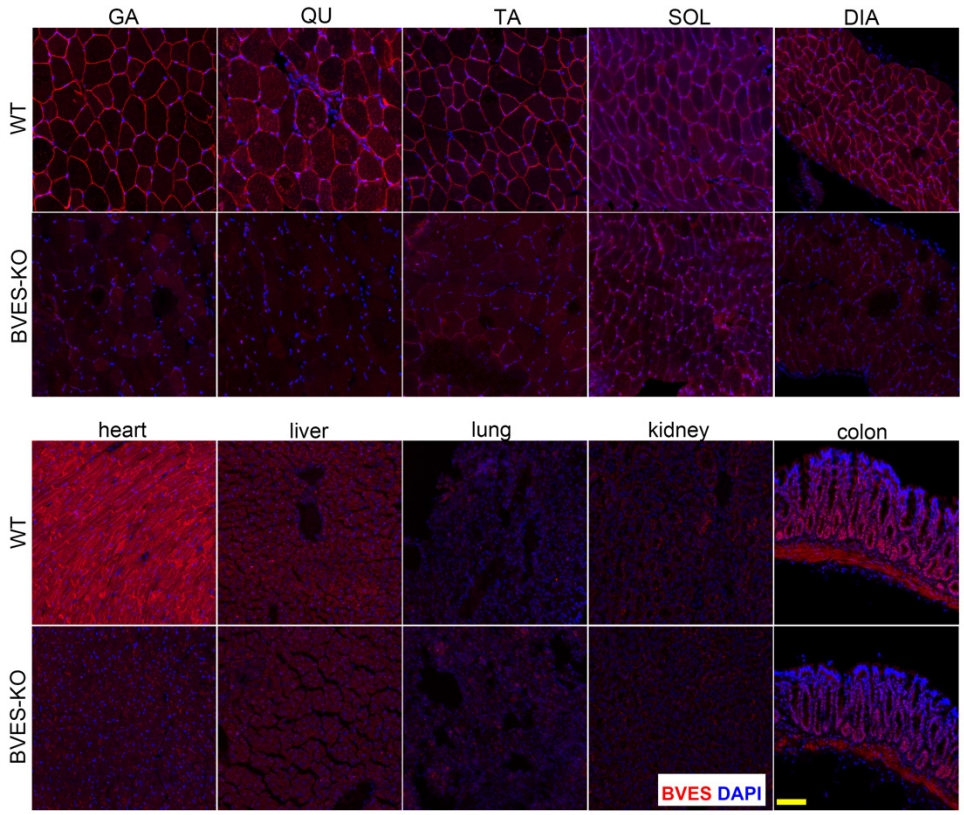

Figure. S3. Endogenous BVES was primarily expressed in skeletal muscles and heart in WT mice. Scale bar: 100 μm.

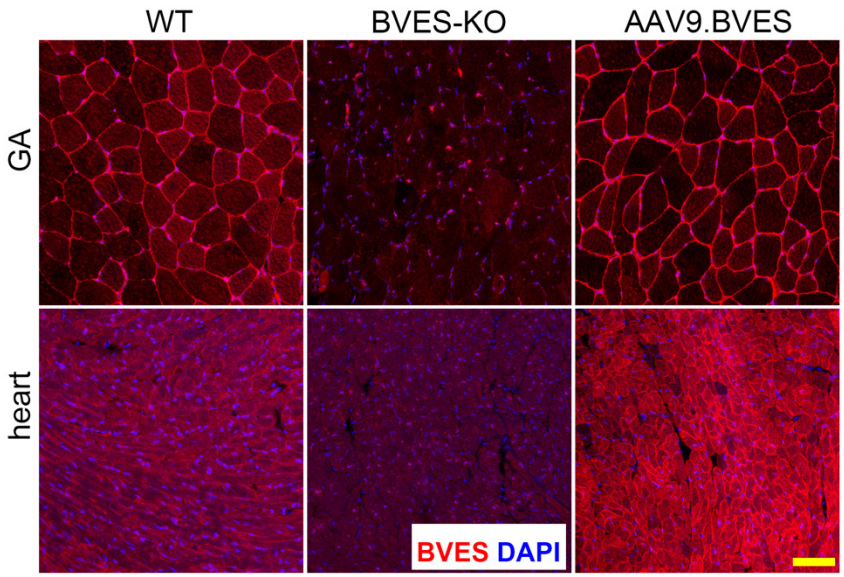

Figure. S4. The BVES transgene showed a similar subcellular localization in skeletal muscle and heat as the endogenous BVES. Scale bar: 100 μm.

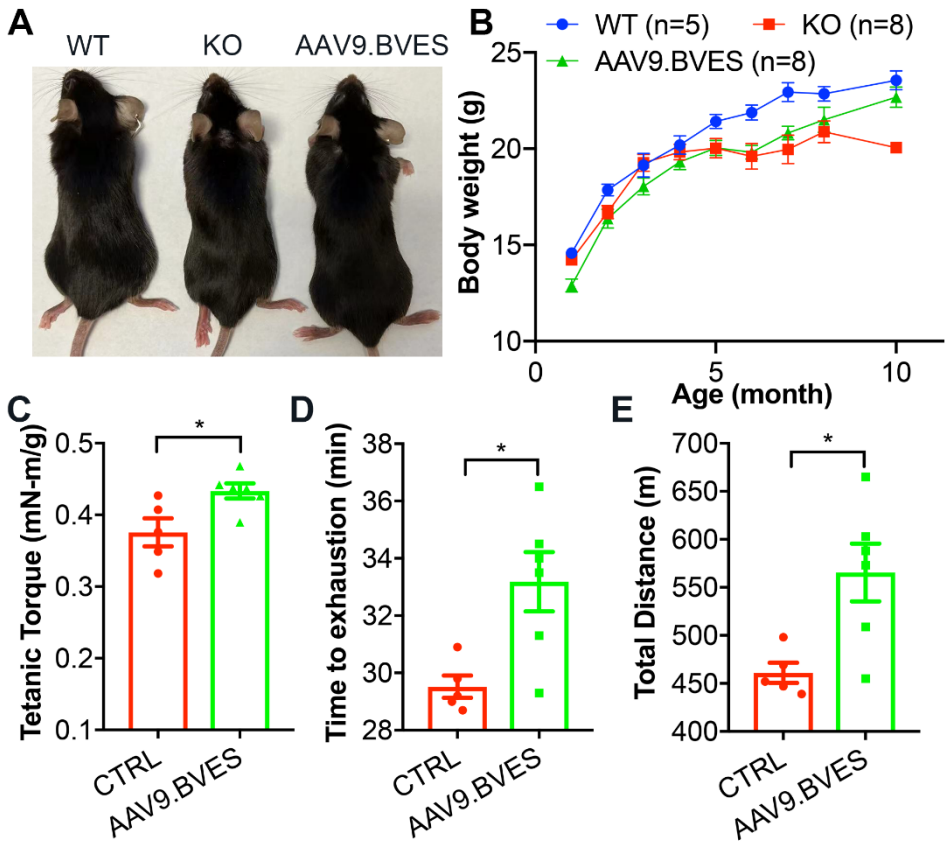

Figure S5. Impact of AAV9.BVES treatment on muscle function and mass in female BVES-KO mice following neonatal IP administration. (A) Representative images of female WT and BVES-KO mice treated with or without AAV9.BVES at 9 months of age. (B) Monthly body weight measurements of female WT and BVES-KO mice treated with or without AAV9.BVES. (C) Tetanic torque measurements of the posterior compartment muscles in female BVES-KO mice (5 months of age) treated with or without AAV9.BVES. (D, E) The running time to exhaustion (D) and total running distance (E) in 6-month-old female BVES-KO mice treated with or without AAV9.BVES determined by the treadmill running test. *P< 0.05.

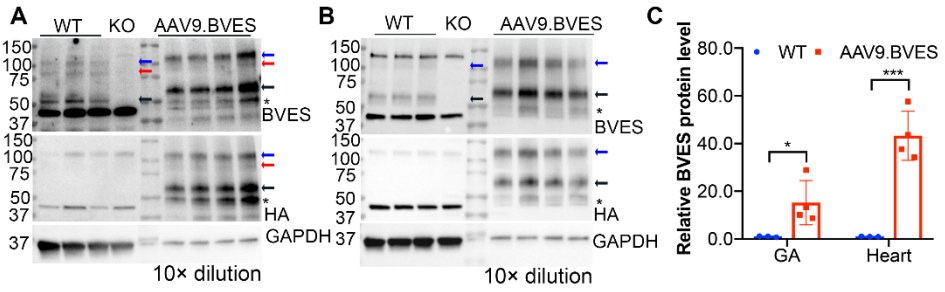

Figure S6. Western blot analysis of BVES transgene expression following tail vein injection of AAV9.BVES in adult male mice. Western blot showed AAV9.BVES was highly expressed in GA muscles (A) and heart (B) in BVES-KO mice with adult administration of AAV9.BVES. Arrows indicate the specific bands of BVES. The quantification was performed using ImageJ (C). **P*< 0.05, ****P*< 0.001.

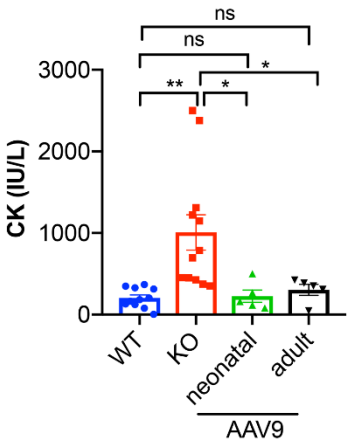

Figure S7. Serum CK measurements in male mice. Serum CK levels in WT and BVES-KO mice treated with or without AAV9.BVES at 9 months age. **P*< 0.05.

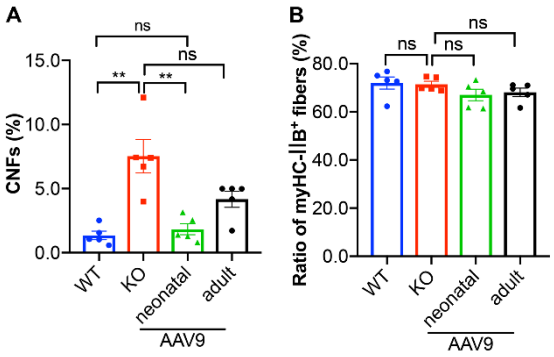

Figure S8. Fiber number count and CNF measurement in skeletal muscles of 9-month-old WT and BVES-KO male mice treated with or without AAV9.BVES. (A) Quantification of CNFs in GA muscles of WT and BVES-KO mice with or without AAV9.BVES at 9 months of age. (B) Quantification of MyHC-IIb muscle fibers versus total fibers from GA muscles of WT and BVES-KO mice with or without AAV9.BVES at 9 months of age. Statistical differences were determined by two-way ANOVA with Turkey's post tests. ns, not significant. $^{**}P < 0.01$; ns, not significant.

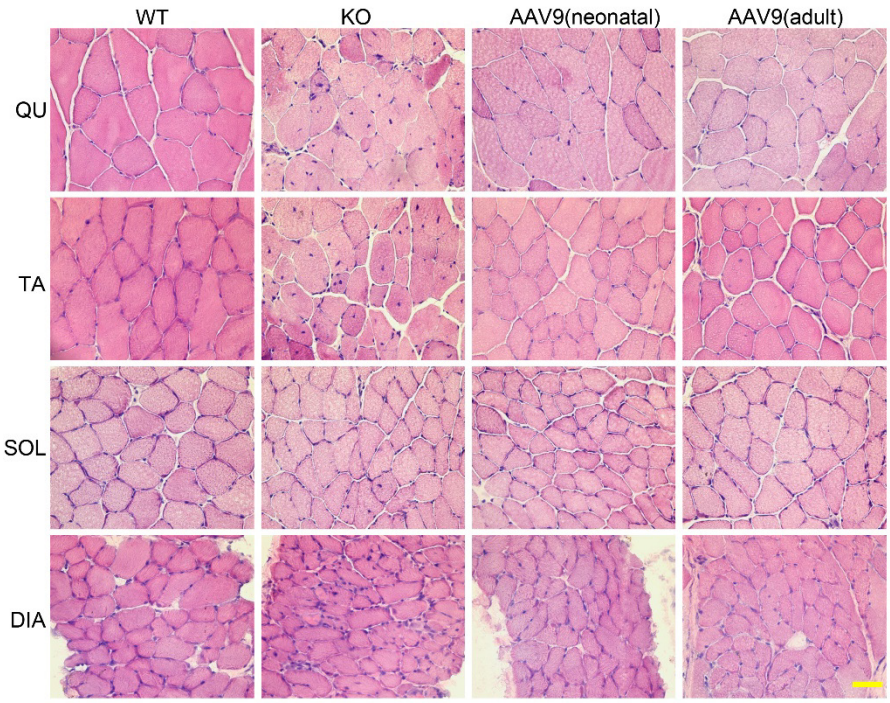

Figure S9. H&E staining of QU, TA, SOL and DIA muscles from WT and BVES-KO mice with or without AAV9.BVES treatment. Scale bar: 100 μm.

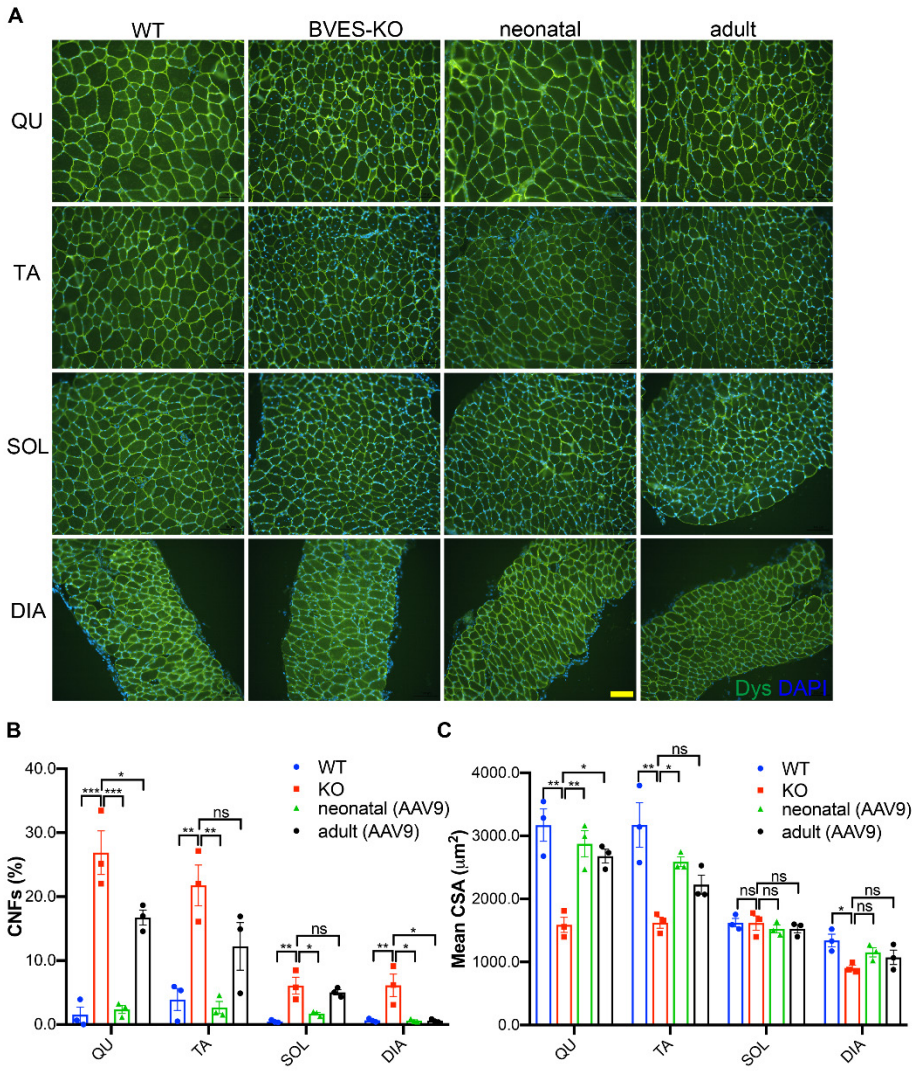

Figure. S10. Systemic AAV9.BVES gene delivery ameliorated the histopathology in various skeletal muscles from male BVES-KO mice. (A) Immunostaining of dystrophin (Dys) in QU, TA, SOL and DIA muscles from WT and BVES-KO mice with or without AAV9.BVES. Scale bar: 100 μm. (B, C) Quantification of CNFs and fiber size area in QU, TA, SOL and DIA muscles from WT and BVES-KO mice with or without AAV9.BVES. Statistical differences were determined by two-way ANOVA with Turkey's post tests. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

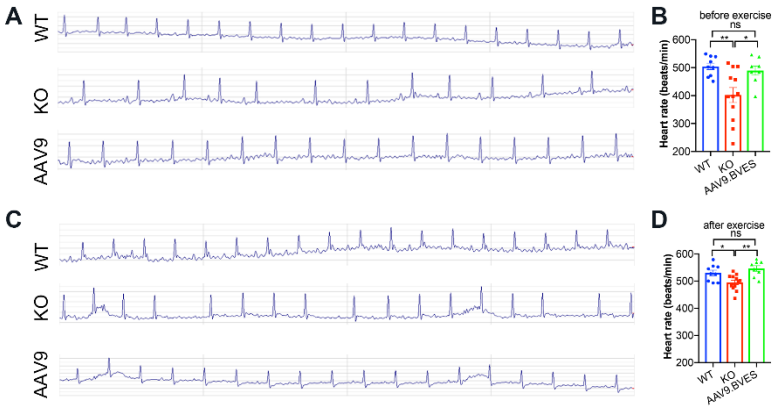

Figure S11. Effects of AAV9.BVES treatment on the heart rate of female BVES-KO mice. (A-D) Representative ECG recordings and heart rate of female WT and BVES-KO mice treated with or without AAV9.BVES before (A, B) and after treadmill running (C, D). *P< 0.05, **P < 0.01. ns, not significant (one-way ANOVA).

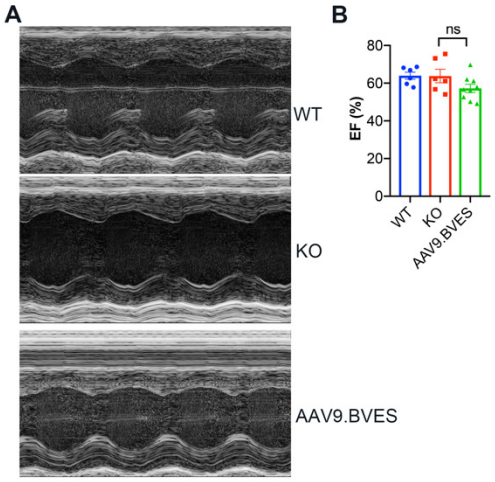

Figure S12. Echocardiography measurement of cardiac function in male BVES-KO mice with or without AAV9.BVES treatment. (**A**) Representative M-mode echocardiographic recording from 9-month-old WT and BVES-KO mice treated with or without AAV9.BVES. (**B**) Ejection fraction showed no significant changes among the three groups of mice.