OMTM, Volume *19*

Supplemental Information

High Levels of Frataxin Overexpression Lead to Mitochondrial and Cardiac Toxicity in Mouse Models

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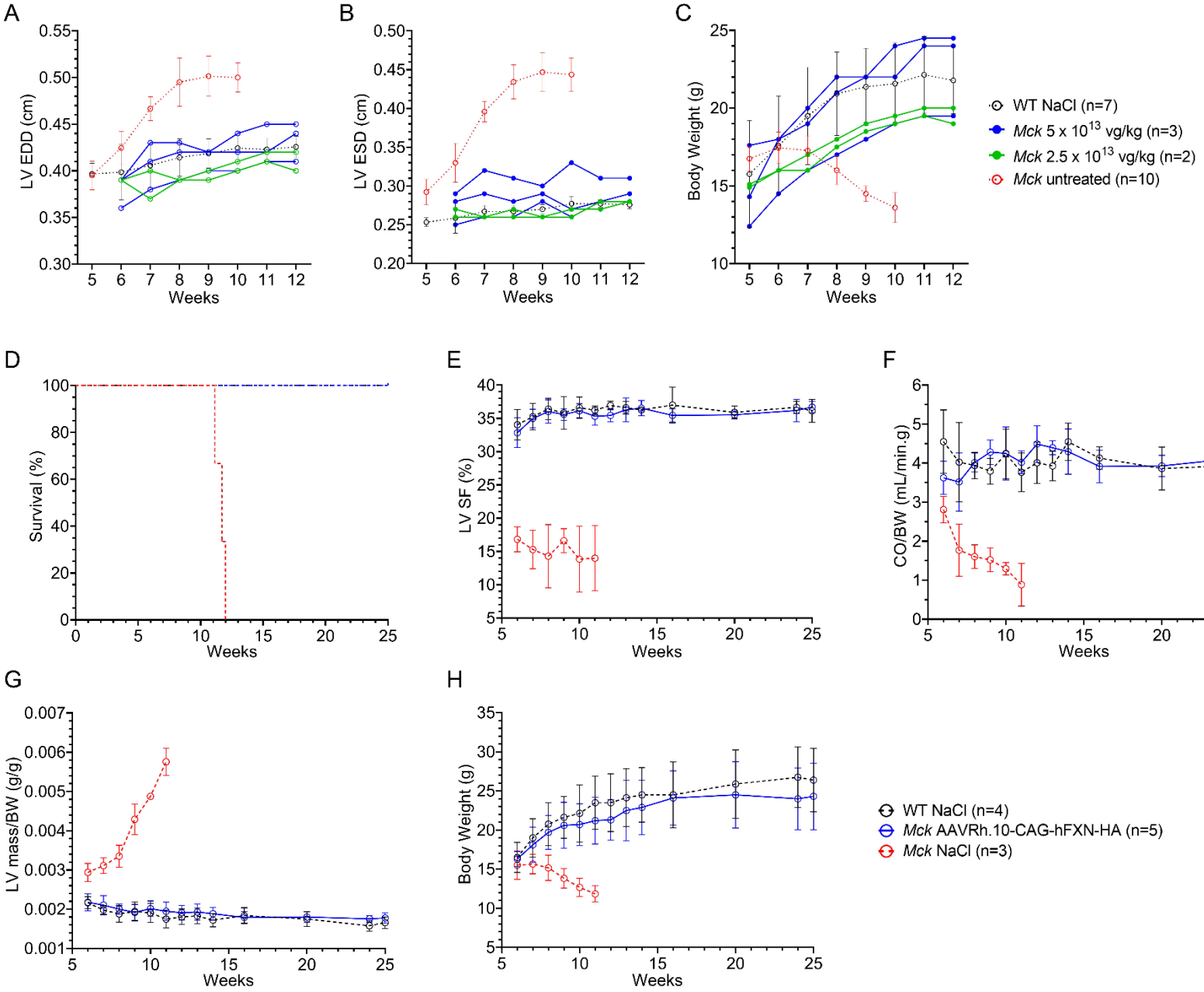

A
LV EDD (cm)
Weeks
B
LV ESD (cm)
Weeks
C
Body Weight (g)
Weeks
WT NaCl (n=7)
Mck 5 x 10^13 vg/kg (n=3)
Mck 2.5 x 10^13 vg/kg (n=2)
Mck untreated (n=10)
D
Survival (%)
Weeks
E
LV SF (%)
Weeks
F
CO/BW (mL/min.g)
Weeks
G
LV mass/BW (g/g)
Weeks
H
Body Weight (g)
Weeks
WT NaCl (n=4)
Mck AAVRh.10-CAG-hFXN-HA (n=5)
Mck NaCl (n=3)

Figure S1. Extended longitudinal echocardiography evaluation of *Mck* mice treated at 5 weeks of age with AAVRh.10-CAG-hFXN-HA vector, up to 25 weeks of age. See also figure 1. **(A-C)** WT mice treated with NaCl (n=7) and *Mck* mice treated at $5x10^{13}$ (n=3) or $2.5x10^{13}$ (n=2) vg/kg and sacrificed at 12 weeks of age for histological and molecular analysis. Data are represented as individual kinetics for treated *Mck* mice. Control WT mice and untreated *Mck* mice are reported as mean ± SD. For untreated *Mck* mice, historical data were plotted. Statistical analyses are reported in Table S2. Figure adapted from Belbellaa et al 2019 Human Molecular Genetics. **(A)** Left ventricle (LV) end-diastole diameter (LV EDD). **(B)** LV end-systole diameter (LV ESD). **(C)** Body weight. **(D-G)** Cohort of mice treated similarly and followed-up until 25 weeks of age. *Mck* mice treated at $5x10^{13}$ vg/kg (n=5), WT (n=4) and *Mck* (n=3) mice treated with NaCl. Data are reported as mean ± SD and statistical analyses are reported in Table S3. (**D**) Survival analysis. Log-rank Mantel-Cox statistical test: WT vs *Mck* AAV, p>0.999; WT vs *Mck* NaCl, p=0.0101; *Mck* AAV vs *Mck* NaCl, p=0.0042. **(E)** LV shortening fraction. **(F)** Cardiac blood output measured at the aorta (CO) and normalized to body weight (BW). **(G)** LV mass normalized to BW. **(H)** Body weight.

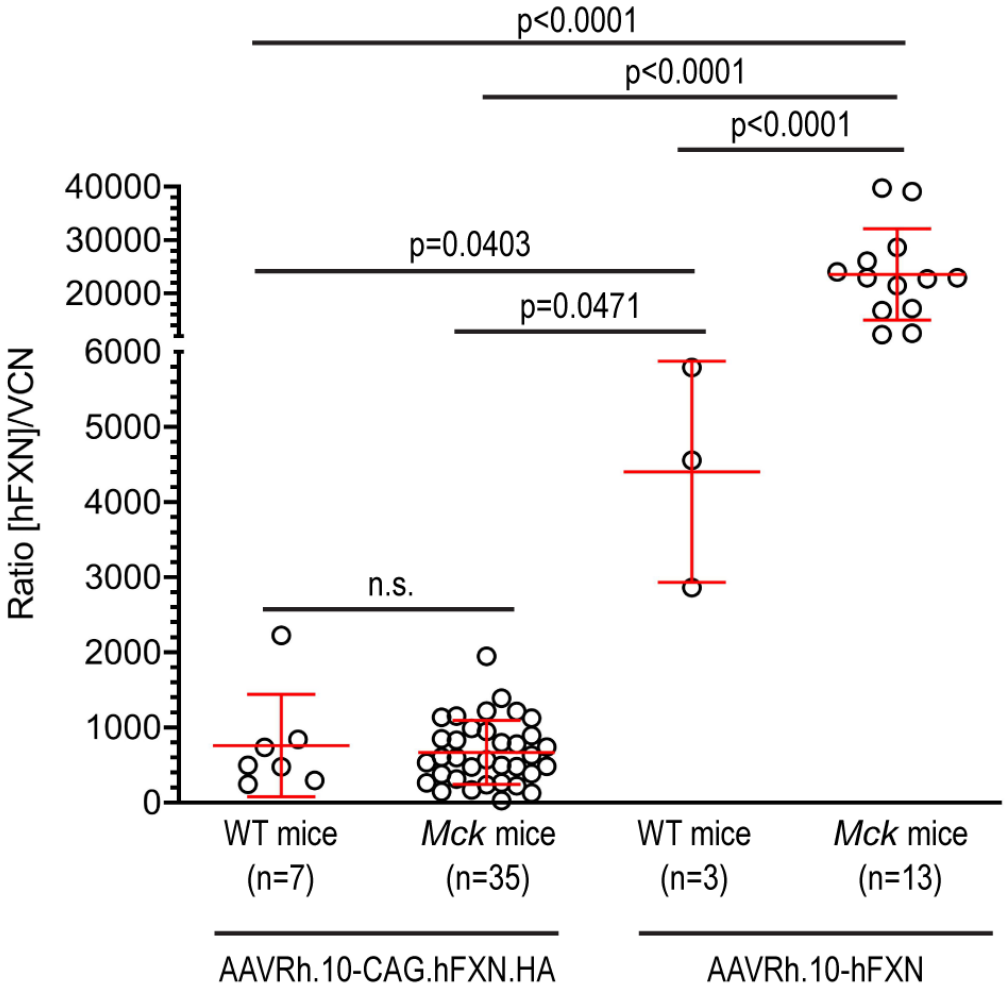


Figure S2. Comparison of AAVRh.10-CAG-hFXN-HA and AAVRh.10-hFXN vectors expression in the heart of WT and *Mck* mice treated at 7 weeks and sacrificed at 15 weeks. Vector biodistribution and expression were quantified in each individual mouse heart. The tissue concentration in human FXN protein was measured by ELISA assay (expressed as ng of FXN per mg of total protein) and normalized by the average vector DNA copies per diploid genome. WT mice (n=7) injected with the AAVRh.10-CAG-hFXN-HA vector, as well as WT mice (n=3) and *Mck* mice (n=13) injected with the AAVRh.10-hFXN, correspond to mice injected for the present study, see Table S1. *Mck* mice (n=35) injected with the AAVRh.10-CAG-hFXN-HA vector, correspond to historical data from our previous dose response study, where mice were treated at 5 or 7 weeks of age, with doses ranging from $5x10^{13}$ down to $1x10^{12}$ vg/kg, see Belbellaa et al 2019 Human Molecular Genetic. Individual datapoint are reported, with mean and SD. Brown-Forsythe and Welch one-way ANOVA statistical test, p values are reported with n.s. p>0.05.

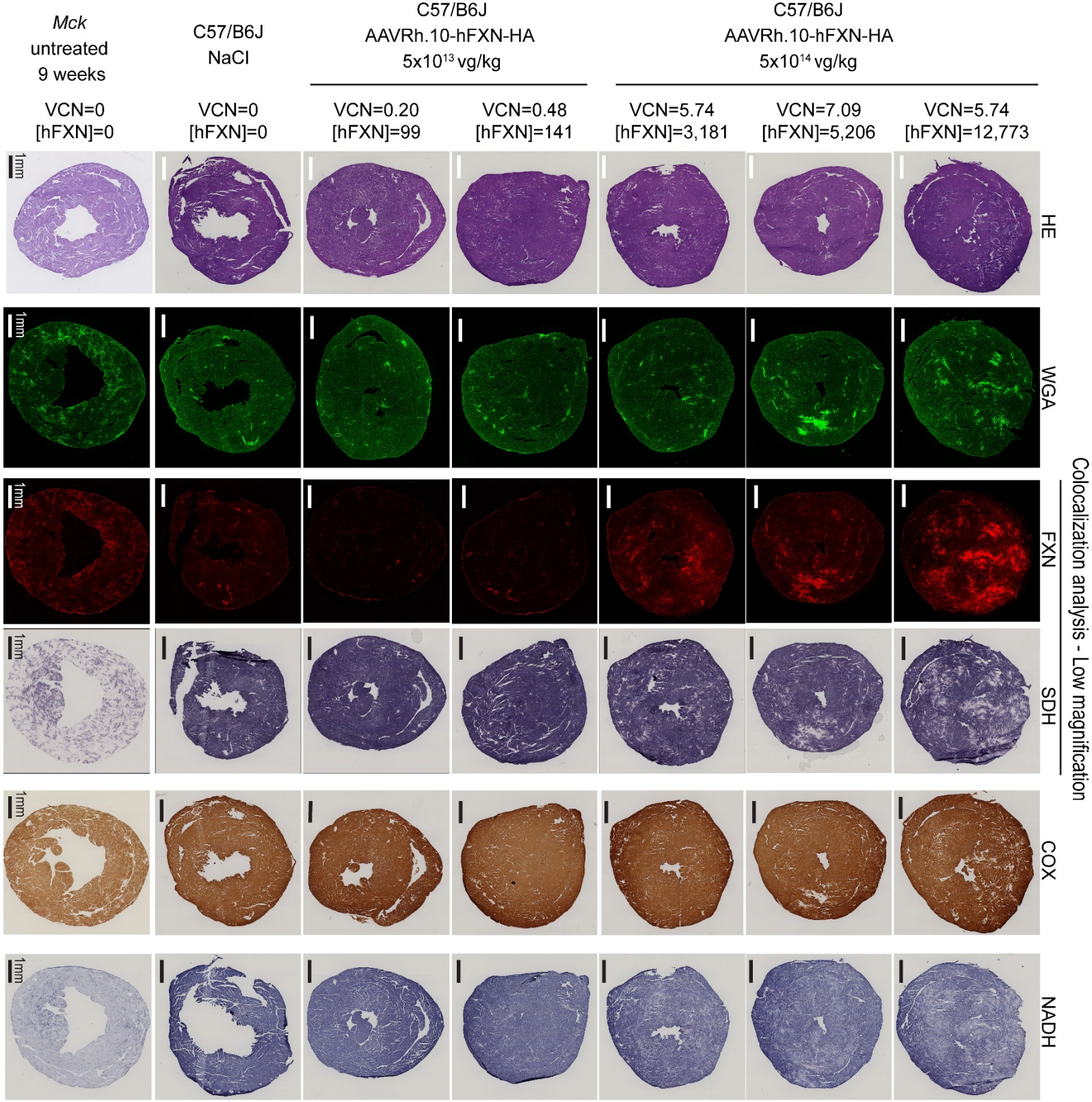

Mck
untreated
9 weeks
VCN=0
[hFXN]=0
C57/B6J
NaCl
VCN=0
[hFXN]=0
C57/B6J
AAVRh.10-hFXN-HA
5x10[13] vg/kg
VCN=0.20
[hFXN]=99
VCN=0.48
[hFXN]=141
C57/B6J
AAVRh.10-hFXN-HA
5x10[14] vg/kg
VCN=5.74
[hFXN]=3,181
VCN=7.09
[hFXN]=5,206
VCN=5.74
[hFXN]=12,773
1mm
HE
WGA
FXN
SDH
COX
NADH
Colocalization analysis - Low magnification

Figure S3. Extended histological analysis and observation at low-magnification of heart tissue section from wild-type C57/B6J mice treated with AAVRh.10-CAG-FXN-HA vector. See also figures 2 and 3. Representative images from the histological analysis of adjacent heart tissue section collected from WT C57/B6J mice treated at 7-weeks of age with vehicle (n=1) or AAVRh.10-CAG-hFXN-HA vector at the dose of $5x10^{14}$ (n=4) or $5x10^{13}$ (n=3) vg/kg, and then sacrificed at 21 weeks of age. For control, we analyzed heart tissue sections from untreated *Mck* mice collected at 9 weeks of age (Table S1). The two left columns correspond to histological analysis of heart fibrosis following staining with hematoxylin-eosin (HE) or wheat germ agglutin conjugated to Alexa488nm (WGA). The two middle columns represent single tissue section and microscopy field, after co-staining of FXN-HA by immunofluorescence and succinate dehydrogenase (SDH) activity by *in-situ* histoenzymatic assay, in order to assess the colocalization of FXN overexpression hotspots and the impairment of SDH enzymatic activity. The two-right columns correspond to *in-situ* histoenzymatic activity assay for cytochrome c oxidase (COX) and NADH-ubiquinone oxidoreductase (NADH). The corresponding dose, VCN and [hFXN] are reported next to each image series. Scale bar, 1mm. Same time exposure used for each labelling series.

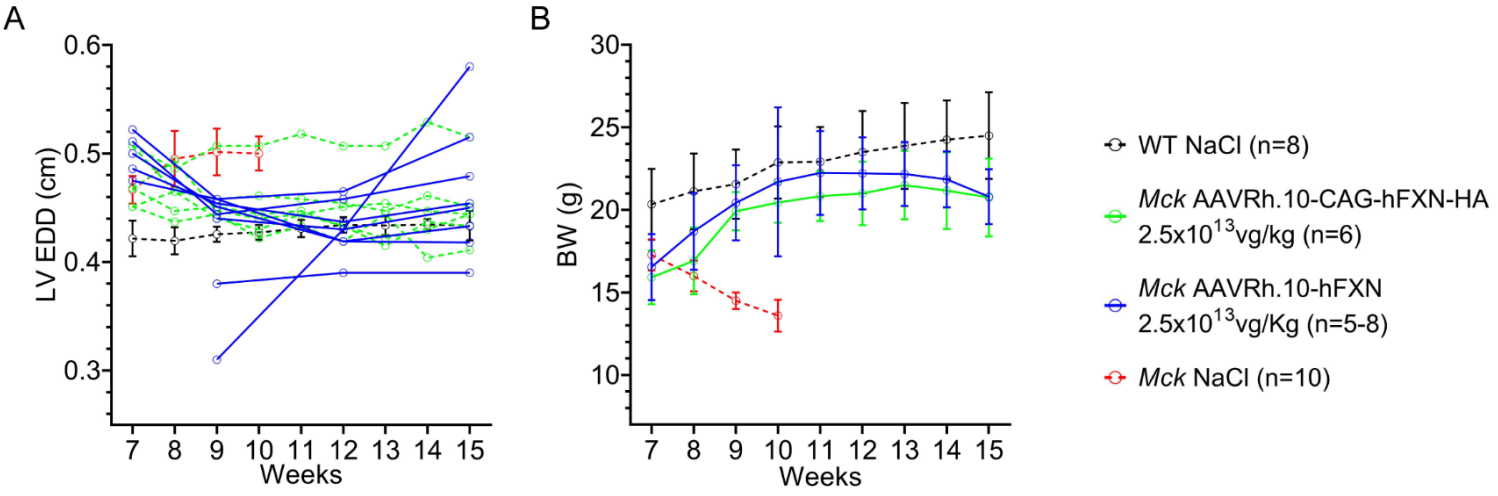
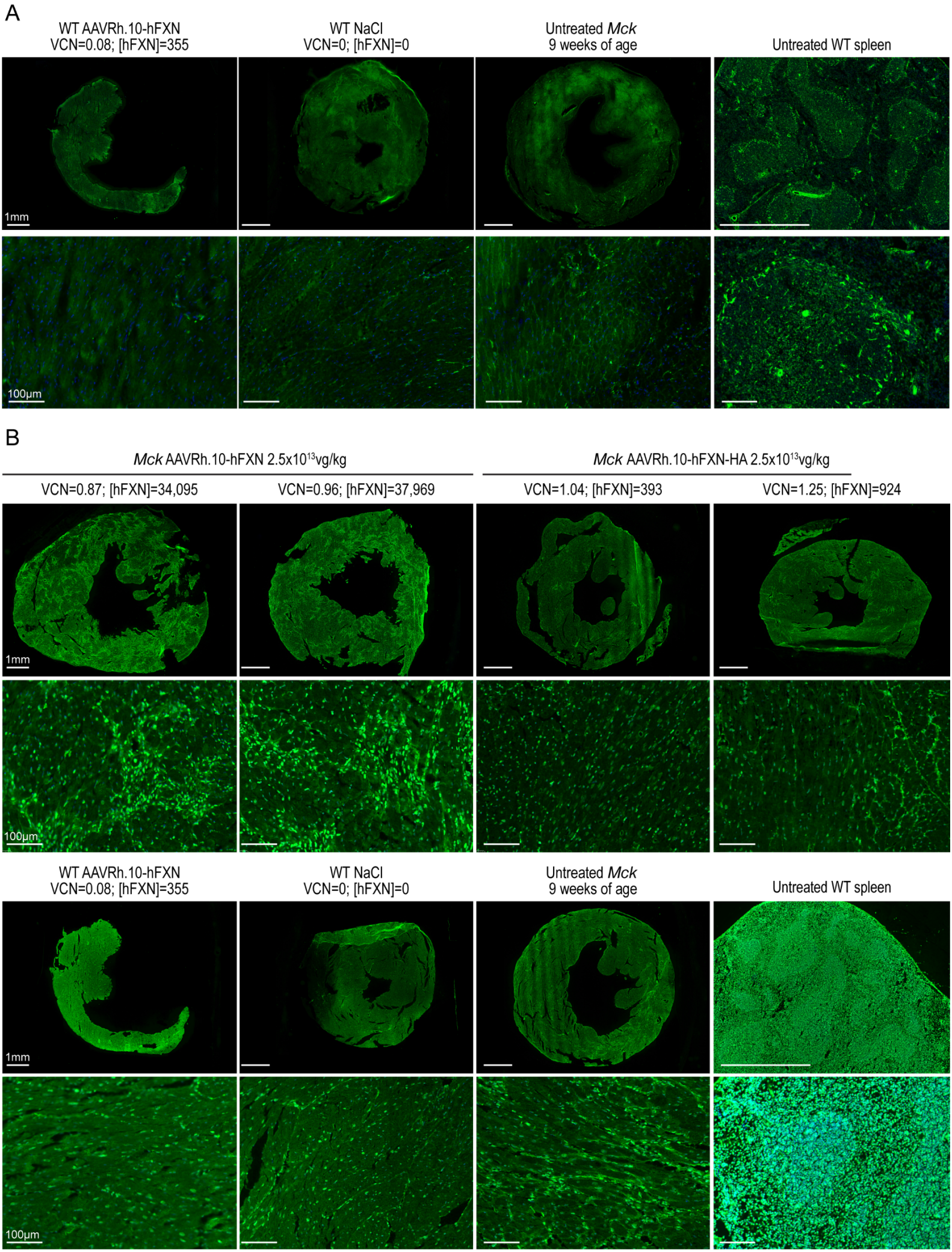


Figure S4. Supplementary longitudinal echocardiography analysis of *Mck* mice treated at 7 weeks of age with AAVRh.10-CAG-hFXN-HA or AAVRh.10-hFXN vector at 2.5x10^{13} vg/kg. See also figure 5. **(A)** Left ventricle (LV) end-diastole diameter (LV EDD). Data are represented as individual kinetics for treated *Mck* mice with AAVRh.10-CAG-hFXN-HA (n=6) or with AAVRh.10-hFXN (n=8) vector. Control NaCl-injected WT (n=8) and *Mck* mice (n=10) are reported as mean ± SD. Statistical analysis is reported in Table S4. **(B)** Body weight (BW) are reported as mean ± SD. For untreated *Mck* mice, historical data were plotted.


A
WT AAVRh.10-hFXN
VCN=0.08; [hFXN]=355
WT NaCl
VCN=0; [hFXN]=0
Untreated *Mck*
9 weeks of age
Untreated WT spleen
1mm
100µm
B
Mck AAVRh.10-hFXN 2.5x10[13]vg/kg
Mck AAVRh.10-hFXN-HA 2.5x10[13]vg/kg
VCN=0.87; [hFXN]=34,095
VCN=0.96; [hFXN]=37,969
VCN=1.04; [hFXN]=393
VCN=1.25; [hFXN]=924
1mm
100µm
WT AAVRh.10-hFXN
VCN=0.08; [hFXN]=355
WT NaCl
VCN=0; [hFXN]=0
Untreated *Mck*
9 weeks of age
Untreated WT spleen
1mm
100µm

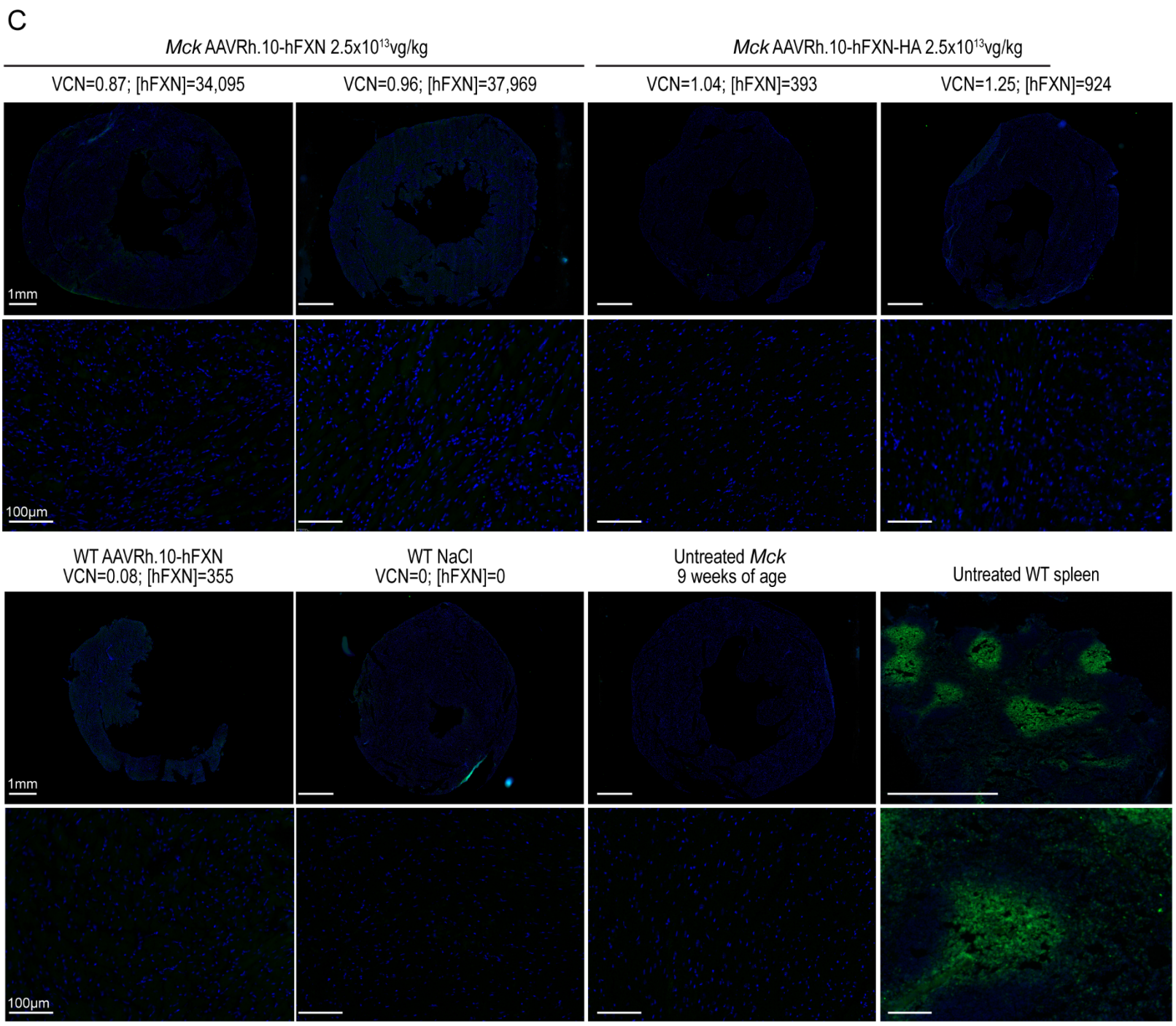
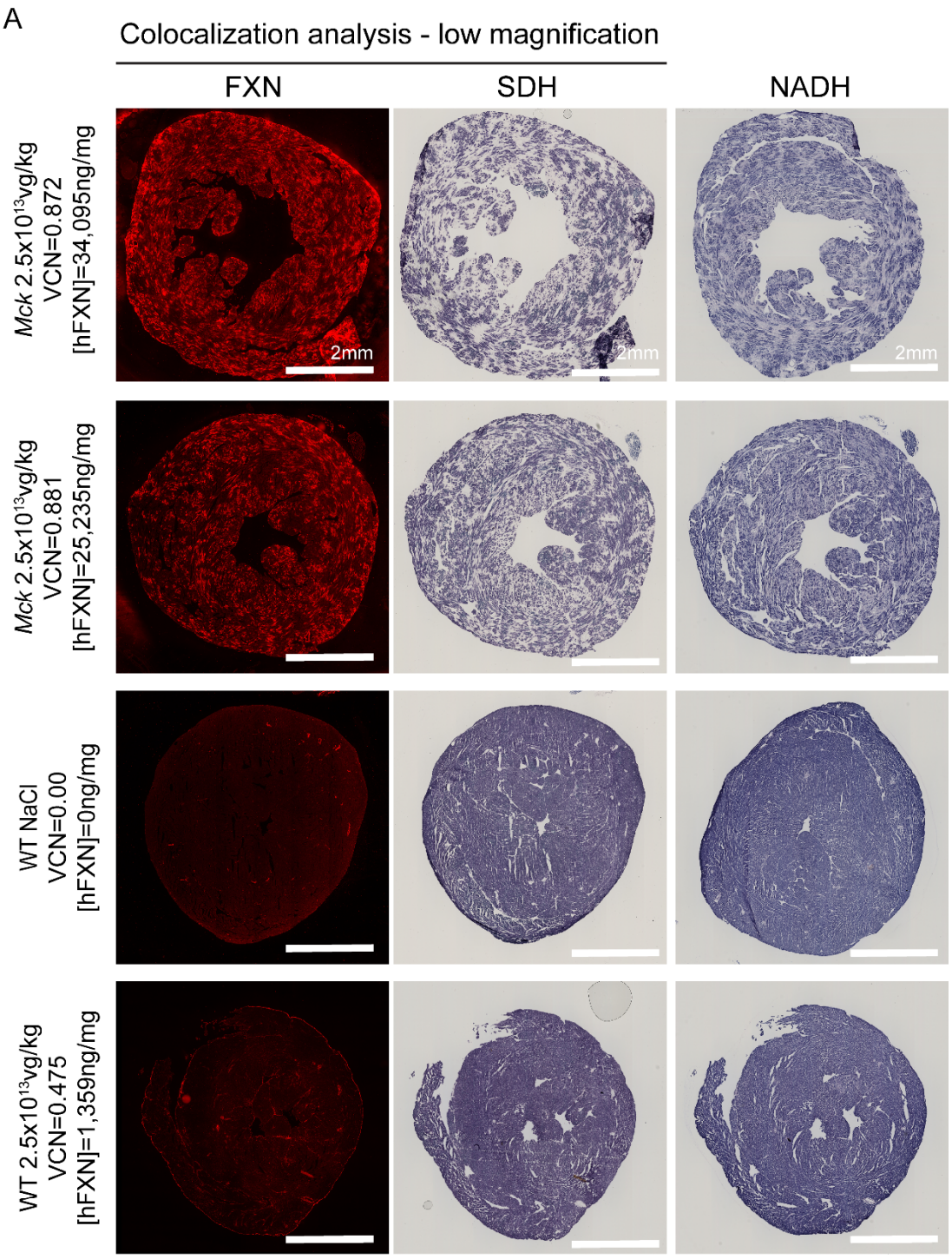


Figure S5. Extended histological analysis of immune cells infiltration in the heart of *Mck* and wild-type mice treated at 7 weeks with AAVRh.10-hFXN or AAVRh.10-CAG-hFXN-HA vector and sacrificed at 15 weeks. See also figure 6. Representative histological analysis on adjacent heart tissue sections from *Mck* mice treated with AAVRh.10-hFXN (n=3) or AAVRh.10-CAG-hFXN-HA (n=3) at $2.5x10^{13}$vg/kg , WT mouse injected with AAVRh.10-hFXN (n=1) at $5x10^{12}$vg/kg, NaCl-injected WT mice (n=3). For control, 9-weeks old untreated *Mck* mice heart and WT mouse spleen (n=1) were also analyzed. **(A)** Immunofluorescence labelling of the monocyte cells marker CD14. **(B)** Immunofluorescence labelling of the leukocyte cells marker CD45. **(C)** Immunofluorescence labelling of the lymphocyte cells marker CD3.


A
Colocalization analysis - low magnification
FXN
SDH
NADH
Mck 2.5x10[13]vg/kg
VCN=0.872
[hFXN]=34,095ng/mg
Mck 2.5x10[13]vg/kg
VCN=0.881
[hFXN]=25,235ng/mg
WT NaCl
VCN=0.00
[hFXN]=0ng/mg
WT 2.5x10[13]vg/kg
VCN=0.475
[hFXN]=1,359ng/mg
2mm

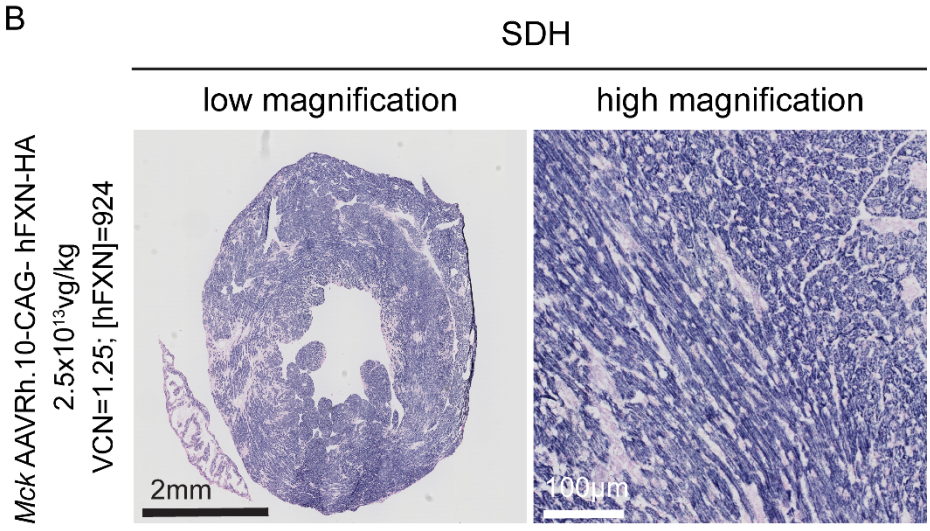

B
SDH
low magnification
high magnification
Mck AAVRh.10-CAG- hFXN-HA
2.5x10[13]vg/kg
VCN=1.25; [hFXN]=924
2mm
100µm

Figure S6. Extended histological analysis of mitochondrial enzymatic activity and observation at low-magnification of heart tissue sections from wild-type and *Mck* mice treated at 7 weeks with AAVRh.10-hFXN or AAVRh.10-CAG-hFXN-HA vector at 2.5x10^{13} vg/kg and sacrificed at 15 weeks. See also figure 7. **(A)** Representative image and histological analysis of adjacent heart tissue sections from *Mck* mice (n=3) and WT mice (n=2) treated with AAVRh.10-hFXN vector and NaCl-injected WT mice (n=2). Left and middle columns represent the single tissue sections and microscopy fields, after co-staining of FXN by immunofluorescence and succinate dehydrogenase (SDH) activity by *in-situ* histoenzymatic assay, in order to assess the colocalization of FXN overexpression hotspots and the impairment of SDH enzymatic activity. Right-column corresponds to *in-situ* histoenzymatic activity assay for NADH-ubiquinone oxidoreductase (NADH). **(B)** *In situ* histoenzymatic assay performed on heart tissue section from a *Mck* mouse treated with AAVRh.10-CAG-hFXN-HA vector and imaged at low and high magnifications. The respective dose, VCN and [hFXN] are reported above each image series. Same time exposure for each labelling series.

Video S1. Echocardiography parasternal short-axis imaging of the left ventricle performed at 21 weeks of age, in wild-type C57/B6J mice treated with AAVRh.10-CAG-FXN-HA vector at 7 weeks of age.

Table S1. Experimental design: **s**ummary of mice age of injection, vector and dose administrated, effective and bioanalytical.

Age of treatment in weeks	Animal groups	Genotype	Treatment/vector	Dose (vg/kg)	Effective	Age of sacrifice in weeks	Bioanalytical
Untreated	A	*Mck* mice	None	N/A	6	9*	Histological analysis
	B				4	8*	Electron microscopy
	C				10 - historical data†	spontaneous death	Echocardiography Survival
5 weeks of age	1	*Mck* mice	AAVRh.10-CAG-hFXN-HA	$5x10^{13}$	3	12§	Echocardiography Vector biodistribution/expression Histological analysis
	2			$2.5x10^{13}$	2		
	3			$5x10^{13}$	5	25‡	Echocardiography
	4		NaCl	N/A	3		
	5	WT mice	NaCl	N/A	7	12§	Echocardiography and histology
	6			N/A	4	25‡	Echocardiography
7 weeks of age	7	*Mck* mice	AAVRh.10-CAG-hFXN-HA	$2.5x10^{13}$	6	22⁋	Echocardiography vector biodistribution/expression Histology analysis Molecular analysis Electron microscopy
	8		AAVRh.10-hFXN	$2.5x10^{13}$	8	22⁋	
	9	WT mice	AAVRh.10-CAG-hFXN-HA	$5x10^{14}$	4	15⁂	
	10		AAVRh.10-CAG-hFXN-HA	$5x10^{13}$	3	15⁂	
	11		AAVRh.10-hFXN	$2.5x10^{13}$	2	15⁂	
	12		AAVRh.10-hFXN	$5x10^{12}$	1	15⁂	
	13		NaCl	N/A	11	15⁂ or 22⁋	

Note: *Mck* and WT mice are on 100% C57/B6J genetic background.
Groups A, B and C correspond to untreated *Mck* mice which were used as controls across the different mice studies.
* untreated *Mck* mice median survival is around 10 weeks of age, so groups A and B were sacrifice at 8 and 9 weeks of age to generate control tissues.
† *Mck* knock-out mice display a very robust and reproducible phenotype as demonstrated previously across several studies, such as Puccio et al 2001 Nature genetics, Seznec et al 2004 Human Molecular Genetics and Perdomini et al 2014 Nature Medicine. To reduce the number of mice used to follow the three-R ethical rules, historical echocardiography *Mck* mice data were used here.
Groups 1, 2 and 5 corresponds to mice originating from our previous dose response study, where mice were treated at 5 or 7 weeks of age, with doses ranging from $5x10^{13}$ down to $1x10^{12}$ vg/kg, see Belbellaa et al 2019 Human Molecular Genetic.
§ Mice sacrificed at 12 weeks of age for histological and molecular analysis. ‡ Mice sacrificed at 25 weeks of age for long term echocardiography follow-up.
⁂ Mice sacrificed at 15 weeks or at 22 weeks of age for histological and molecular analysis.

Table S2. Statistical analysis of echocardiography measurements presented in Figures 1D-F and S1A-C.
Mck mice treated at 5 weeks of age with AAVRh.10-CAG-hFXN-HA vector at $5x10^{13}$ or $2.5x10^{13}$ vg/kg and sacrificed at 12 weeks of age. One-way ANOVA analysis, Mixed-effects model (REML), no assumption of sphericity, $\alpha = 0.05$.

Left ventricle shortening fraction			
Fixed effect (type III) **<0.0001**	F (DFn, DFd) F (1.135, 9.079) = 49.42	Geisser-Greenhouse's epsilon = 0.3783	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
WT NaCl (n=7) vs. *Mck* $5x10^{13}$ vg/kg (n=3)	4.250	1.743 to 6.757	**0.0044**
WT NaCl (n=7) vs. *Mck* $2.5x10^{13}$ vg/kg (n=2)	1.869	0.02753 to 3.711	**0.0471**
WT NaCl (n=7) vs. *Mck* untreated (n=10)	18.82	10.45 to 27.19	**0.0016**
Mck $5x10^{13}$ vg/kg (n=3) vs. *Mck* $2.5x10^{13}$ vg/kg (n=2)	-2.381	-4.643 to -0.1192	**0.0406**
Mck $5x10^{13}$ vg/kg (n=3) vs. *Mck* untreated (n=10)	14.57	4.878 to 24.26	**0.0123**
Mck $2.5x10^{13}$ vg/kg (n=2) vs. *Mck* untreated (n=10)	16.95	7.139 to 26.76	**0.0074**
Cardiac output normalized to body weight			
Fixed effect (type III) **<0.0001**	F (DFn, DFd) F (1.590, 12.72) = 90.72	Geisser-Greenhouse's epsilon = 0.5299	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
WT NaCl (n=7) vs. *Mck* $5x10^{13}$ vg/kg (n=3)	0.09299	-0.4230 to 0.6090	0.9207
WT NaCl (n=7) vs. *Mck* $2.5x10^{13}$ vg/kg (n=2)	0.2953	-0.2019 to 0.7926	0.2669
WT NaCl (n=7) vs. *Mck* untreated (n=10)	2.727	1.868 to 3.586	**0.0003**
Mck $5x10^{13}$ vg/kg (n=3) vs. *Mck* $2.5x10^{13}$ vg/kg (n=2)	0.2024	-0.08695 to 0.4917	0.1728
Mck $5x10^{13}$ vg/kg (n=3) vs. *Mck* untreated (n=10)	2.634	1.374 to 3.894	**0.0036**
Mck $2.5x10^{13}$ vg/kg (n=2) vs. *Mck* untreated (n=10)	2.432	1.454 to 3.410	**0.0019**
Left ventricle mass normalized to body			
Fixed effect (type III) = **0.0014**	F (DFn, DFd) F (1.015, 8.123) = 22.10	Geisser-Greenhouse's epsilon = 0.3385	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
WT NaCl (n=7) vs. *Mck* $5x10^{13w}$vg/kg (n=3)	7.256e-005	-2.800e-005 to 0.0001731	0.1575
WT NaCl (n=7) vs. *Mck* $2.5x10^{13}$vg/kg (n=2)	8.208e-005	-2.791e-006 to 0.0001670	0.0571
WT NaCl (n=7) vs. *Mck* untreated (n=10)	-0.001551	-0.003006 to -9.562e-005	0.0395
Mck $5x10^{13}$vg/kg (n=3) vs. *Mck* $2.5x10^{13}$vg/kg (n=2)	9.524e-006	-9.986e-005 to 0.0001189	0.9895
Mck $5x10^{13}$vg/kg (n=3) vs. *Mck* untreated (n=10)	-0.001624	-0.002941 to -0.0003064	**0.0249**
Mck $2.5x10^{13}$vg/kg (n=2) vs. *Mck* untreated (n=10)	-0.001633	-0.003034 to -0.0002318	**0.0302**
Left ventricle end diastole diameter			
Fixed effect (type III) = **0.0023**	F (DFn, DFd) F (1.074, 6.086) = 24.40	Geisser-Greenhouse's epsilon = 0.3580	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
WT NaCl (n=7) vs. *Mck* $5x10^{13}$vg/kg (n=3)	0.000	-0.01115 to 0.01115	>0.9999
WT NaCl (n=7) vs. *Mck* $2.5x10^{13}$vg/kg (n=2)	0.01476	0.005639 to 0.02388	**0.0056**
WT NaCl (n=7) vs. *Mck* untreated (n=10)	-0.05041	-0.09929 to -0.001542	**0.0446**
Mck $5x10^{13}$vg/kg (n=3) vs. *Mck* $2.5x10^{13}$vg/kg (n=2)	0.01476	-0.0006284 to 0.03015	0.0589
Mck $5x10^{13}$vg/kg (n=3) vs. *Mck* untreated (n=10)	-0.05041	-0.09703 to -0.003798	**0.0387**
Mck $2.5x10^{13}$vg/kg (n=2) vs. *Mck* untreated (n=10)	-0.06518	-0.1239 to -0.006452	**0.0355**
Left ventricle end systole diameter			
Fixed effect (type III) = **0.0016**	F (DFn, DFd) F (1.010, 5.721) = 31.31	Geisser-Greenhouse's epsilon = 0.3365	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
WT NaCl (n=7) vs. *Mck* $5x10^{13}$vg/kg (n=3)	-0.01839	-0.02474 to -0.01205	**0.0002**
WT NaCl (n=7) vs. *Mck* $2.5x10^{13}$vg/kg (n=2)	0.001131	-0.008187 to 0.01045	0.9729
WT NaCl (n=7) vs. *Mck* untreated (n=10)	-0.1221	-0.2032 to -0.04113	**0.0097**
Mck $5x10^{13}$vg/kg (n=3) vs. *Mck* $2.5x10^{13}$vg/kg (n=2)	0.01952	0.008714 to 0.03033	**0.0032**
Mck $5x10^{13}$vg/kg (n=3) vs. *Mck* untreated (n=10)	-0.1037	-0.1842 to -0.02330	**0.0213**

Table S3. Statistical analysis of echocardiography measurements presented in Figure S1D-H.
Mck mice treated at 5 weeks of age with AAVRh.10-CAG-hFXN-HA vector at $5x10^{13}$vg/kg and sacrificed at 25 weeks of age. One-way ANOVA analysis, Mixed-effects model (REML), no assumption of sphericity, $\alpha = 0.05$.

Left ventricle shortening fraction			
Fixed effect (type III) **<0.0001**	F (1.122, 16.28) = 1093	Geisser-Greenhouse's epsilon = 0.5612	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
WT NaCl (n=4) vs. *Mck* AAV $5x10^{13}$vg/kg (n=5)	0.5254	0.06266 to 0.9881	**0.0263**
WT NaCl (n=4) vs. *Mck* NaCl (n=3)	21.00	18.49 to 23.50	**<0.0001**
Mck AAV $5x10^{13}$vg/kg (n=5) vs. *Mck* NaCl (n=3)	20.47	17.76 to 23.18	**<0.0001**
Cardiac output normalized to body weight			
Fixed effect (type III) **<0.0001**	F (0.9670, 14.02) = 107.1	Geisser-Greenhouse's epsilon = 0.4835	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
WT NaCl (n=4) vs. *Mck* AAV $5x10^{13}$vg/kg (n=5)	0.01580	-0.2946 to 0.3262	0.9899
WT NaCl (n=4) vs. *Mck* NaCl (n=3)	2.435	1.925 to 2.945	**<0.0001**
Mck AAV $5x10^{13}$vg/kg (n=5) vs. *Mck* NaCl (n=3)	2.419	1.411 to 3.427	**0.0013**
Left ventricle mass normalized to body			
Fixed effect (type III) **<0.0001**	F (DFn, DFd) F (1.015, 8.123) = 22.10	Geisser-Greenhouse's epsilon = 0.3385	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
WT NaCl (n=4) vs. *Mck* AAV $5x10^{13}$vg/kg (n=5)	-9.278e-005	-0.0001489 to -3.670e-005	**0.0022**
WT NaCl (n=4) vs. *Mck* NaCl (n=3)	-0.002223	-0.003625 to -0.0008206	**0.0083**
Mck AAV $5x10^{13}$vg/kg (n=5) vs. *Mck* NaCl (n=3)	-0.002130	-0.003489 to -0.0007711	**0.0087**
Body weight			
Fixed effect (type III) = **0.0003**	F (1.024, 8.700) = 33.02	Geisser-Greenhouse's epsilon = 0.5118	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
WT NaCl (n=4) vs. *Mck* AAV $5x10^{13}$vg/kg (n=5)	1.456	0.9015 to 2.010	**<0.0001**
WT NaCl (n=4) vs. *Mck* NaCl (n=3)	8.899	3.487 to 14.31	**0.0071**
Mck AAV $5x10^{13}$vg/kg (n=5) vs. *Mck* NaCl (n=3)	7.443	2.839 to 12.05	**0.0076**

Table S4. Statistical analysis of echocardiography measurements presented in Figures 5I-L and S4A-B.
Mck mice treated at 7 weeks of age with AAVRh.10-CAG-hFXN-HA vector or AAVRh.10-hFXN vector at $2.5x10^{13}$vg/kg. One-way ANOVA analysis, Mixed-effects model (REML), no assumption of sphericity, $\alpha = 0.05$.

Table S4. Statistical analysis of echocardiography measurements in *Mck* mice treated at 7 weeks of age with AAVRh.10-CAG-hFXN-HA vector or AAVRh.10-hFXN vector at $2.5x10^{13}$vg/kg. One-way ANOVA analysis, Mixed-effects model (REML), no assumption of sphericity, $\alpha = 0.05$

Left ventricle shortening fraction			
Fixed effect (type III) = **0.0112**	F (DFn, DFd): F (0.7212, 3.366) = 27.54	Geisser-Greenhouse's epsilon = 0.2404	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Mck AAVRh.10-hFXN (n=8) vs. *Mck* AAVRh.10-CAG-hFXN-HA (n=6)	-1.992	-9.261 to 5.278	0.6098
Mck AAVRh.10-hFXN (n=8) vs. *Mck* untreated (n=10)	14.00	-125.7 to 153.7	0.4786
Mck AAVRh.10-hFXN (n=8) vs. WT NaCl (n=8)	-9.949	-24.29 to 4.392	0.1251
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. *Mck* untreated (n=10)	16.00	-4.653 to 36.64	0.0960
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. WT NaCl (n=8)	-7.957	-14.23 to -1.683	**0.0153**
Mck untreated (n=10) vs. WT NaCl (n=8)	-23.95	-27.96 to -19.95	**0.0003**
Cardiac output normalized to body weight			
Fixed effect (type III) = **0.0112**	F (DFn, DFd): F (0.7212, 3.366) = 27.54	Geisser-Greenhouse's epsilon = 0.2404	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Mck AAVRh.10-hFXN (n=8) vs. *Mck* AAVRh.10-CAG-hFXN-HA (n=6)	-0.4267	-1.736 to 0.8823	0.5016
Mck AAVRh.10-hFXN (n=8) vs. *Mck* untreated (n=10)	2.016	-18.42 to 22.45	0.4847
Mck AAVRh.10-hFXN (n=8) vs. WT NaCl (n=8)	-0.6183	-2.992 to 1.756	0.6401
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. *Mck* untreated (n=10)	2.443	0.3170 to 4.569	**0.0344**
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. WT NaCl (n=8)	-0.1916	-1.029 to 0.6456	0.8813
Mck untreated (n=10) vs. WT NaCl (n=8)	-2.635	-3.164 to -2.105	**0.0006**
Left ventricle mass normalized to body			
Fixed effect (type III) = **0.0138**	F (DFn, DFd): F (0.7034, 3.282) = 24.91	Geisser-Greenhouse's epsilon = 0.2345	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Mck AAVRh.10-hFXN (n=8) vs. *Mck* AAVRh.10-CAG-hFXN-HA (n=6)	0.0009792	4.837e-005 to 0.001910	**0.0437**
Mck AAVRh.10-hFXN (n=8) vs. *Mck* untreated (n=10)	0.001687	-6.490e-006 to 0.003381	**0.0505**
Mck AAVRh.10-hFXN (n=8) vs. WT NaCl (n=8)	-0.0004650	-0.02119 to 0.02026	0.9513
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. *Mck* untreated (n=10)	0.0007082	0.0002797 to 0.001137	**0.0033**
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. WT NaCl (n=8)	-0.001444	-0.003638 to 0.0007495	0.1412
Mck untreated (n=10) vs. WT NaCl (n=8)	-0.002152	-0.003301 to -0.001004	**0.0087**
Left ventricle end diastole diameter			
Fixed effect (type III) = **0.0132**	F (DFn, DFd): F (0.7051, 5.170) = 15.30	Geisser-Greenhouse's epsilon = 0.2350	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Mck AAVRh.10-hFXN (n=8) vs. *Mck* AAVRh.10-CAG-hFXN-HA (n=6)	-0.001386	-0.05710 to 0.05433	0.9992
Mck AAVRh.10-hFXN (n=8) vs. *Mck* untreated (n=10)	-0.03596	-0.9011 to 0.8292	0.8200
Mck AAVRh.10-hFXN (n=8) vs. WT NaCl (n=8)	0.02581	-0.05129 to 0.1029	0.4848
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. *Mck* untreated (n=10)	-0.03457	-0.09014 to 0.02099	0.1603
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. WT NaCl (n=8)	0.02720	0.01198 to 0.04241	**0.0020**
Mck untreated (n=10) vs. WT NaCl (n=8)	-0.06177	-0.09496 to -0.02858	**0.0088**
Left ventricle end systole diameter			
Fixed effect (type III) = **0.0132**	F (DFn, DFd): F (0.7051, 5.170) = 15.30	Geisser-Greenhouse's epsilon = 0.2350	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Mck AAVRh.10-hFXN (n=8) vs. *Mck* AAVRh.10-CAG-hFXN-HA (n=6)	-0.001386	-0.05710 to 0.05433	0.9992
Mck AAVRh.10-hFXN (n=8) vs. *Mck* untreated (n=10)	-0.03596	-0.9011 to 0.8292	0.8200
Mck AAVRh.10-hFXN (n=8) vs. WT NaCl (n=8)	0.02581	-0.05129 to 0.1029	0.4848
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. *Mck* untreated (n=10)	-0.03457	-0.09014 to 0.02099	0.1603
Mck AAVRh.10-CAG-hFXN-HA (n=6) vs. WT NaCl (n=8)	0.02720	0.01198 to 0.04241	**0.0020**
Mck untreated (n=10) vs. WT NaCl (n=8)	-0.06177	-0.09496 to -0.02858	**0.0088**

Table S5. Primers sequence.

Gene	Forward	Reverse
18s	ACCGCAGCTAGGAATAATGGAA	CCTCCGACTTTCGTTCTTGATT
Hprt	GTAATGATCAGTCAACGGGGGAC	CCAGCAAGCTTGCAACCTTAACCA
Fxn	ATGGCGTGCTCACCATTAAG	GGCCAATGAAGACAAGTCCA
FXN	AGAGGAAACGCTGGACTCTT	ACGCTTAGGTCCACTGGATG
Col1a1	TCACCTACAGCACCCTTGTG	GTCCGAATTCCTGGTCTGG
Col3a1	TCAAGGCTGAAGGAAACAGC	GGGTAGTCTCATTGCCTTGC
Tgf · 1	GGAGAGCCCTGGATACCAAC	CAACCCAGGTCCTTCCTAAA
Il1b	AGCTATGGCAACTGTTCCTGA	CTGCCACAGCTTCTCCACA
Il6	GTGACAACCACGGCCTTC	ACAACTCTTTTCTCATTTCCACGA
Tnf ·	TCAGTTCTATGGCCCAGACCC	GTCTTTGAGATCCATGCCGTT
Nppa	TCGTCTTGGCCTTTTGGCT	TCCAGGTGGTCTAGCAGGTTCT
Nppb	AAGTCCTAGCCAGTCTCCAGA	GAGCTGTCTCTGGGCCATTTC
Asns	ATTACGACAGTTCGGGCATC	TCTCAGTTCGAGACCGTGTC
Mthfd2	AATTTGGGCTTTGCAGTGAC	ACACTCCCAAAGAGCAGCTG
Ddit3	CCAGAATAACAGCCGGAACC	ATCCTCATACCAGGCTTCCA
Trib3	CGCTTTGTCTTCAGCAACTGT	TCATCTGATCCAGTCATCACG
Fgf21	ACCTCTACACAGATGACGACCA	AGAAACCTAGAGGCTTTGACACC
Gdf15	GCTGCTACTCCGCGTCAACC	CTACCCGTAAGCGCAGTTCC