DZHK
DEUTSCHES ZENTRUM FÜR
HERZ-KREISLAUF-FORSCHUNG E.V.

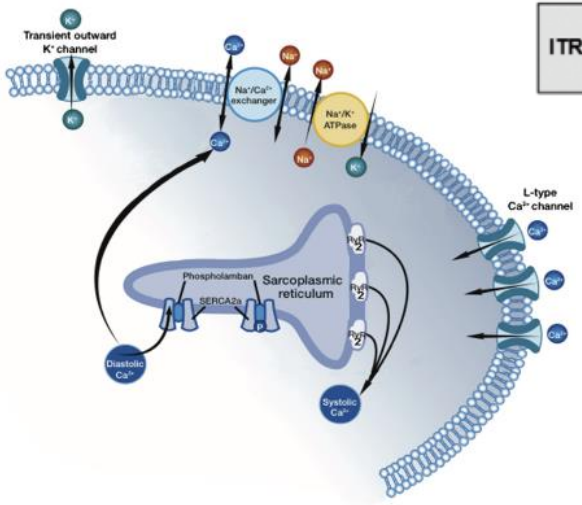
Else Kröner Fresenius Stiftung

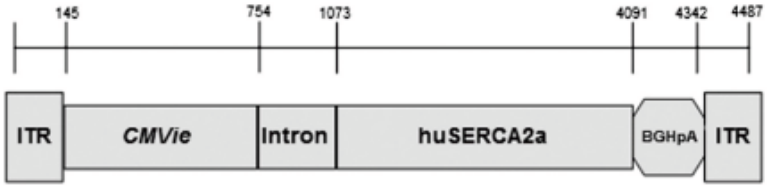
TECHNISCHE UNIVERSITÄT MÜNCHEN

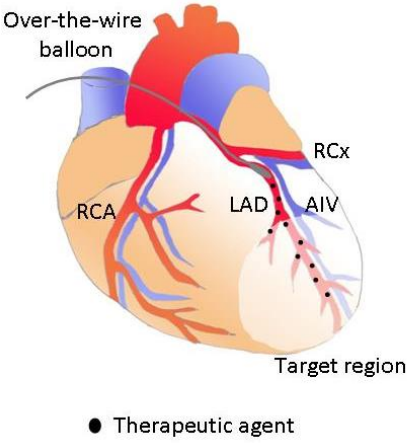
Gene therapy and gene editing – Examples of successful translation

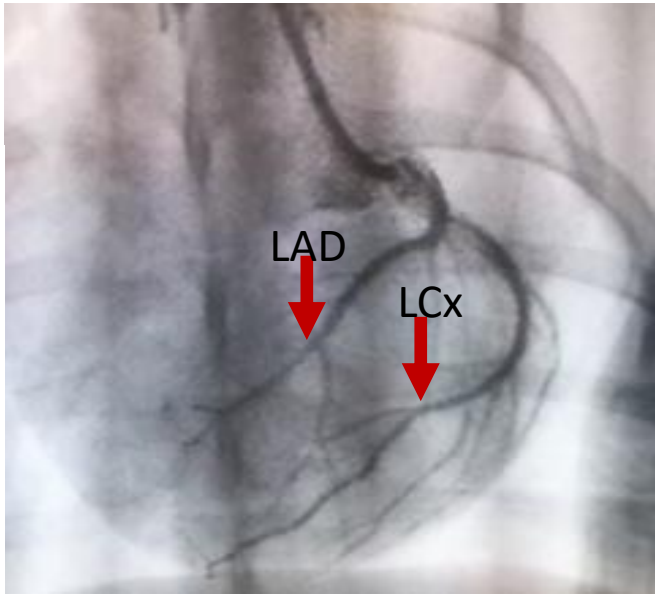
C. Kupatt
Medizinische Klinik und Poliklinik I
Klinikum rechts der Isar, München

AAV1-Serca as heart failure gene therapy







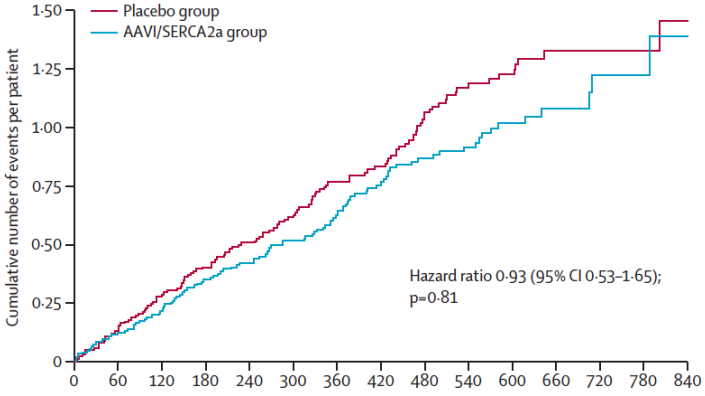


Greenberg et al.,
JACC Heart Failure 2014

Hinkel & Kupatt, Cardiovasc Res 2012

Phase II Study of AAV1-SERCA2a in Heart Failure

CUPID 2: 250 Patients



Number at risk

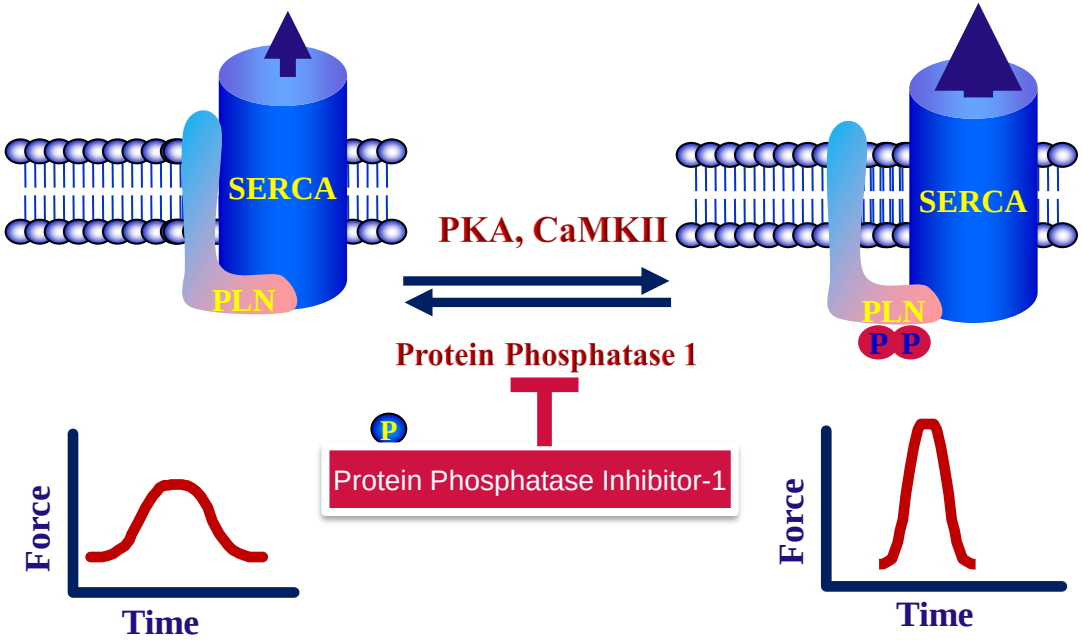
Placebo group	122	122	120	119	114	108	103	83	72	57	45	27	11	9	4
AAVI/SERCA2a group	121	119	115	113	109	104	102	81	67	55	40	24	13	6	1

Greenberg et al., Lancet 2016

Viral Uptake in Animal Models of Heart Failure

Animals	Virus	Delivery	ss copies of viral DNA/μg DNA	% Infected Cardiac Cells
Mice	AAV9.SERCA2a	Intravenous	42,000	~75%
Rats	AAV9.SERCA2a	Intravenous	30,000	~70%
Pigs	AAV1.SERCA2a	Intracoronary	8,000	~30%
Sheep	AAV6.SERCA2a AAV1.SERCA2a	Intracoronary Surgical, MCARD	9,000 13,000	~33% ~42%
Humans	AAV1.SERCA2a	CUPID AGENT HF SERCA-LVAD	<561 ND <80	1% 0 <0.2%

PP1 Is Regulated by Inhibitor-1

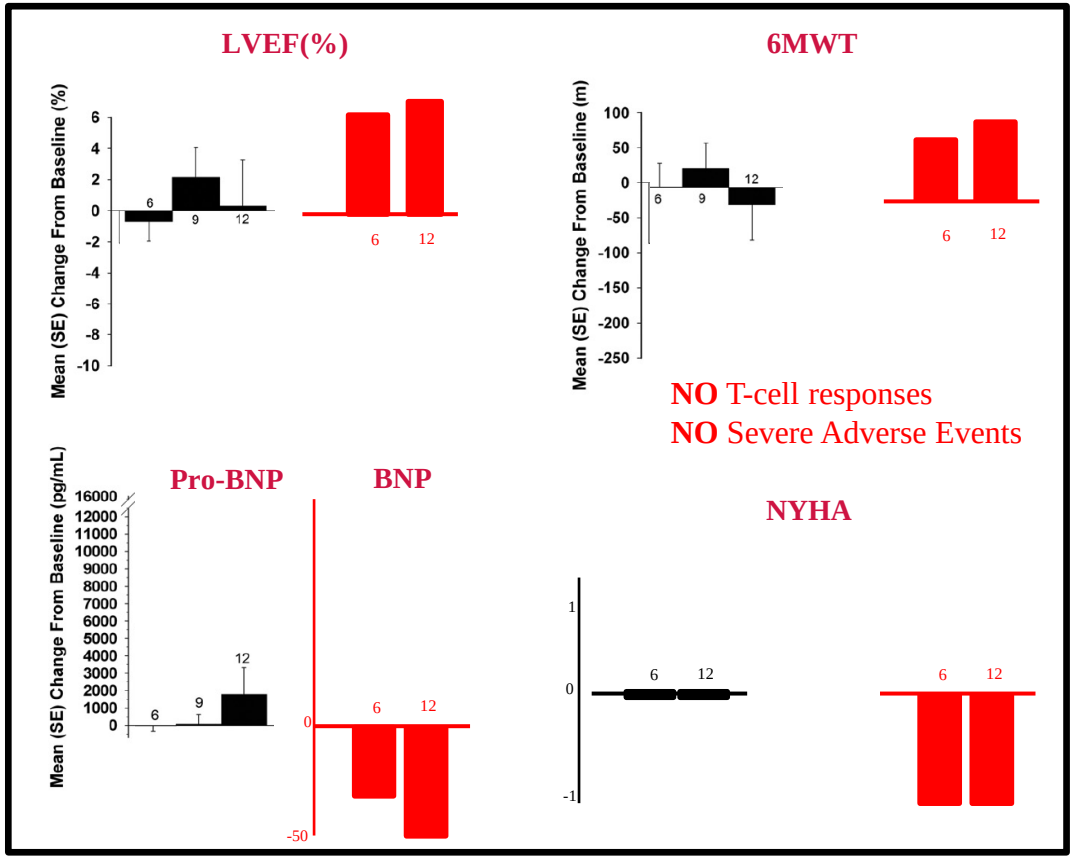


Significant Clinical Efficacy of Dose-Escalation

10^{13} vg/patient in CUPID Trial by Celladon

$3x10^{13}$vg/patient in Cohort A of MUSIC Trial by Sardocor

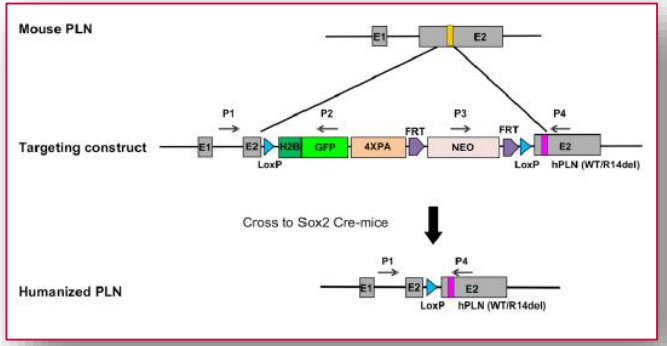
Further escalation in Cohort B to be performed in 1Q2023

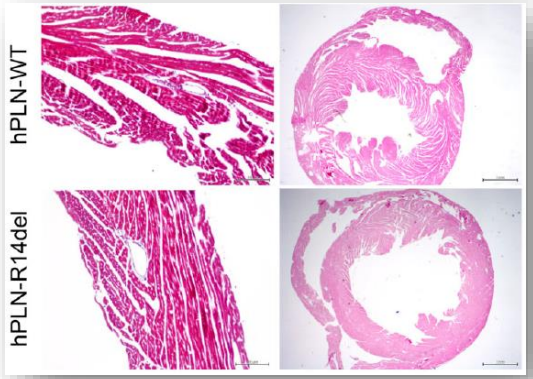


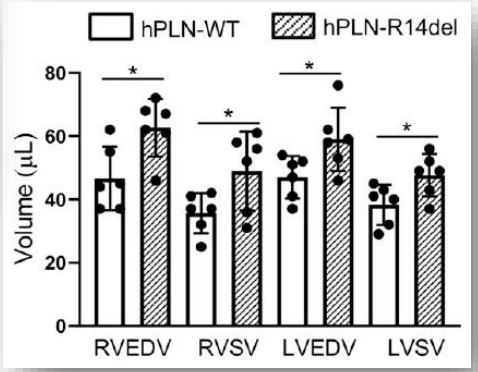
Successfully gene-edited or gene-supplemented genetic cardiomyopathies

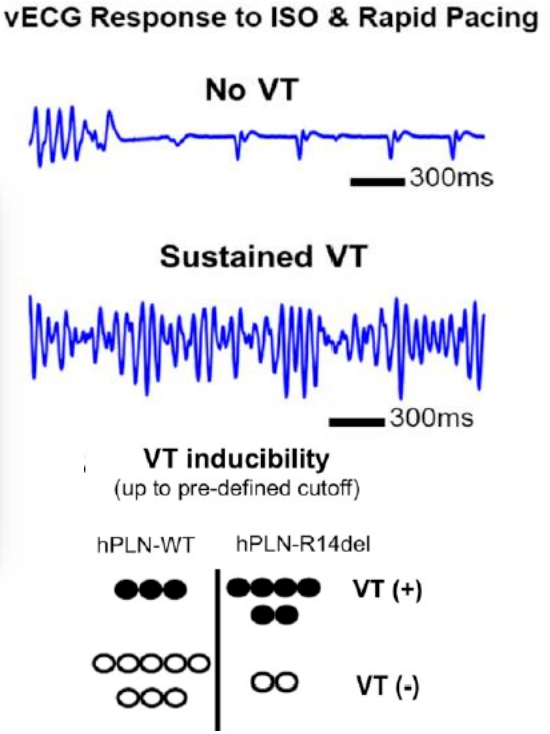
- **Hypertrophy**
  - *MYH7*
  - *MYBPC*
- **Dilatation**
  - *RBM20*
  - *Titin*
  - *DMD*
  - *PLN* R14del
- **ARVC / AVC**
  - *PKP 2*

Phospholamban R14 del mutation



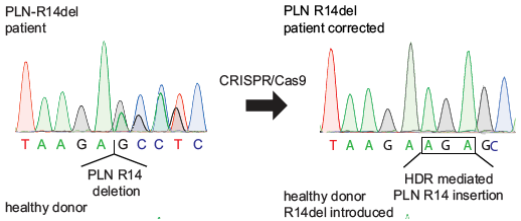


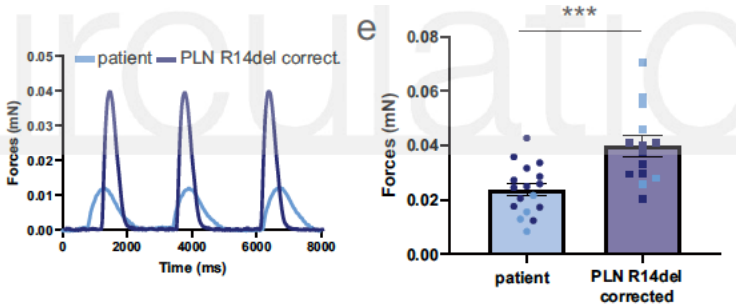




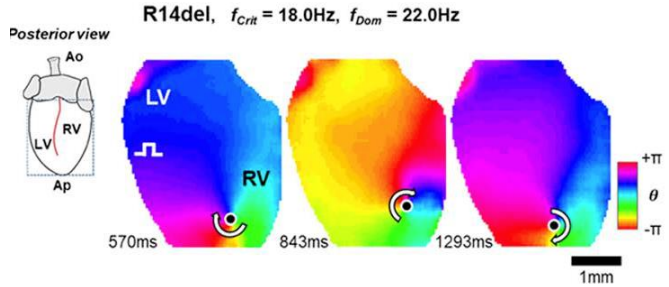
Dave et al., Cardiovasc Res 2022

Reversion of PLN R14del arrhythmogenic phenotype by Cas9 editing in vivo (mouse)

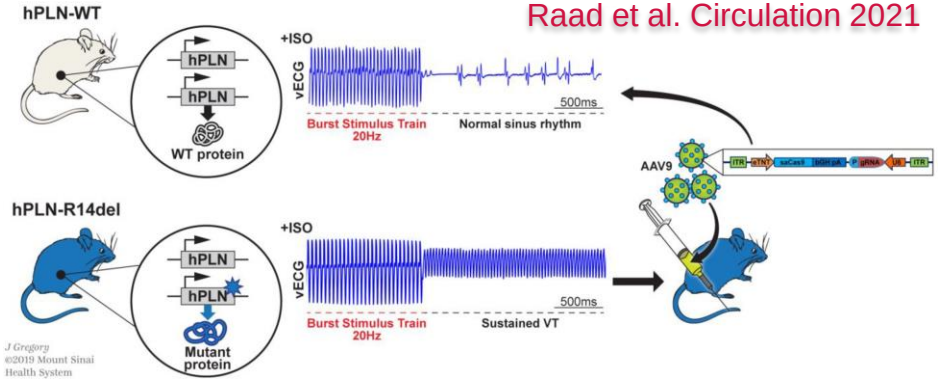




Feyen et al., Circulation 2021

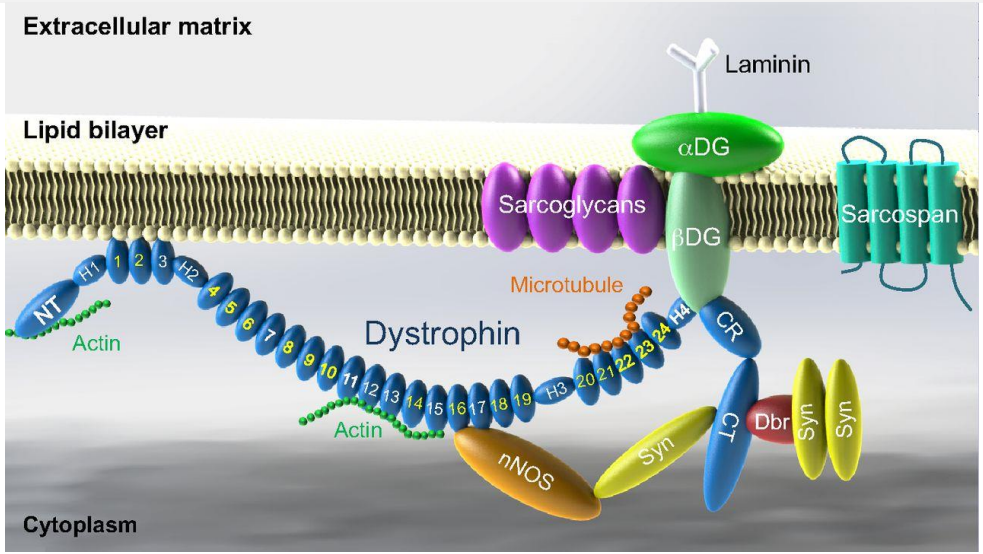


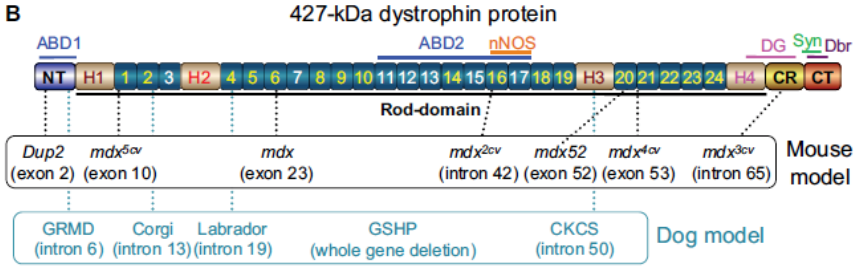
Raad et al. Circulation 2021



Dave et al., Cardiovasc Research 2022

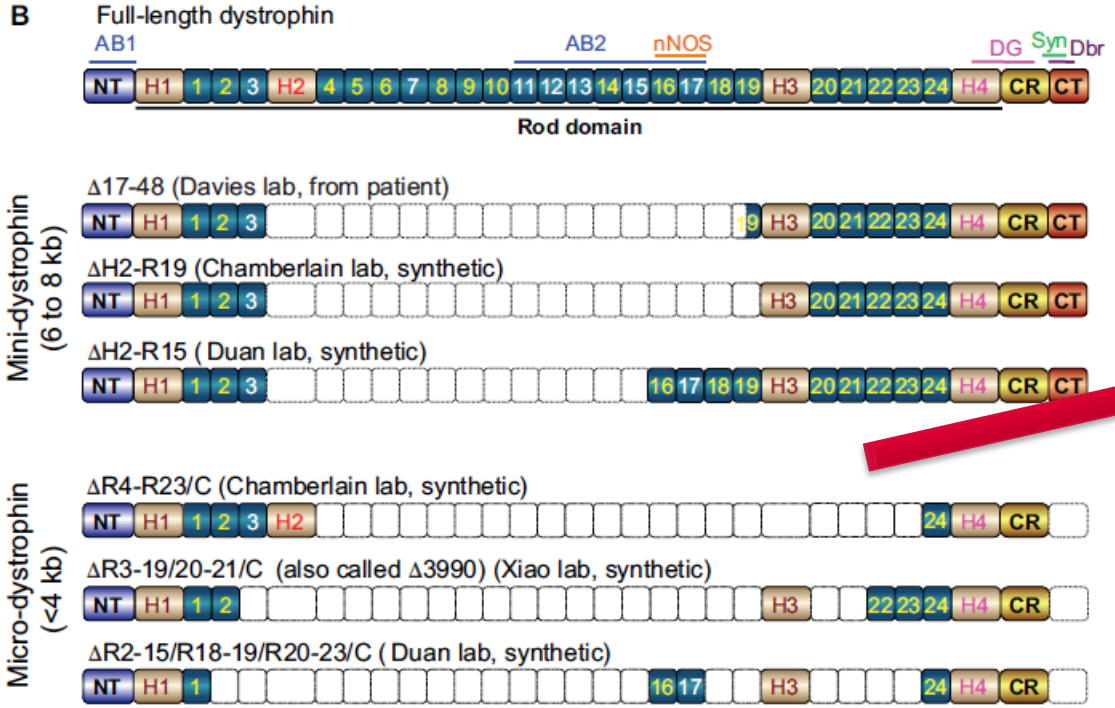
Dystrophin



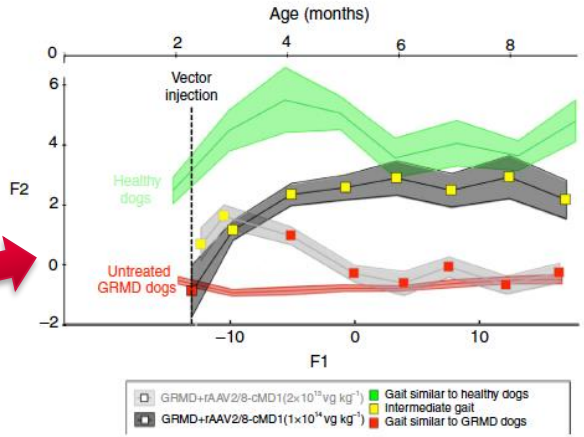


McGreevy et al., 2015

Mini- and microdystrophins



i.v. application of AAV8-Spc5.12-cMD1

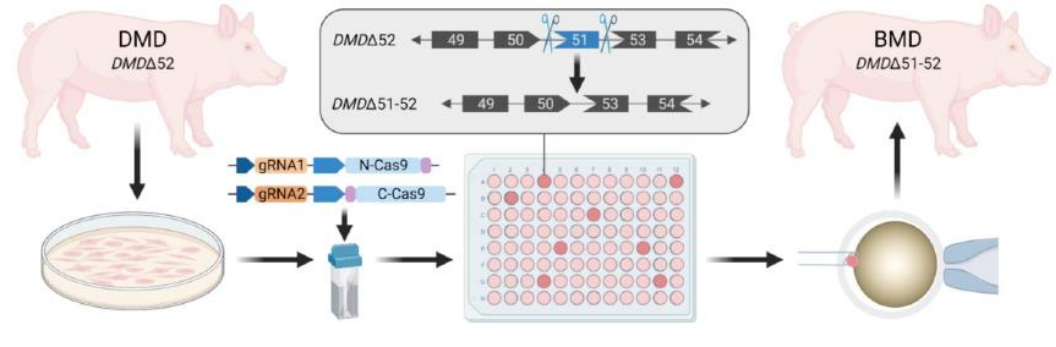


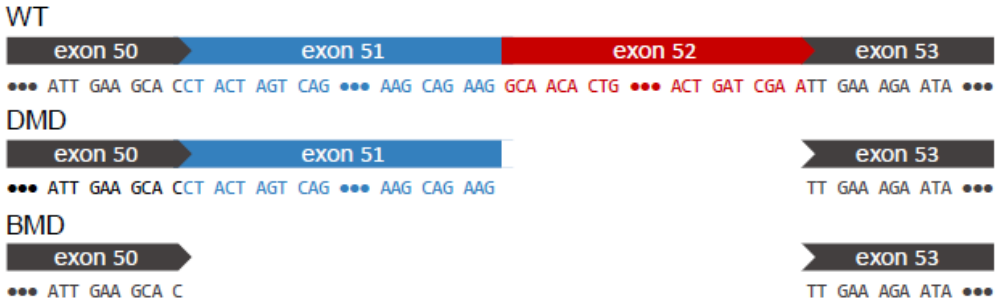
Now in clinical trial (Sarepta)

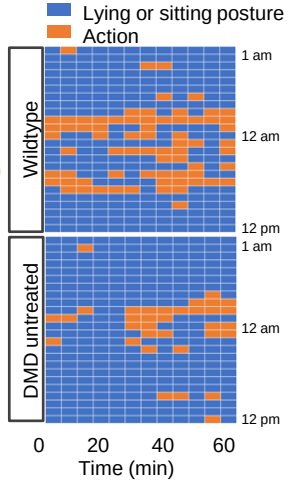
Greevy et al., Dis. Mod. Mechan. 2015

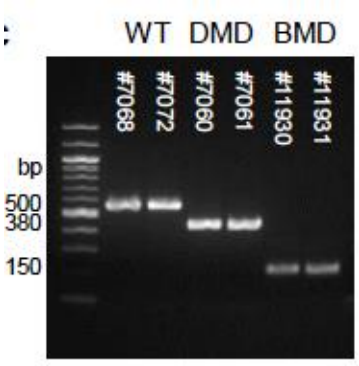
Le Guiner et al., Nat Comms 2017

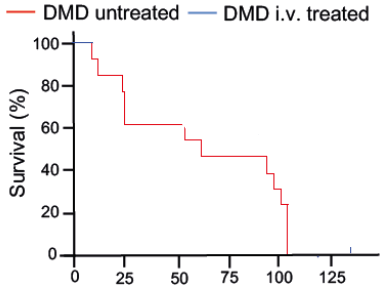
Genotype of DMD and BMD pigs



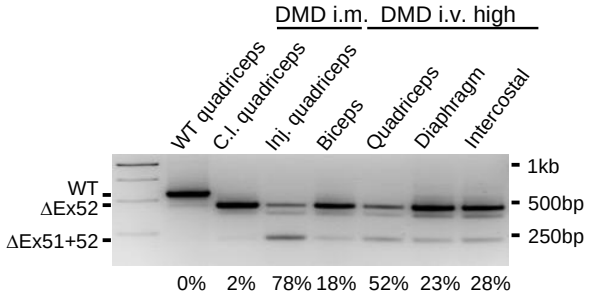


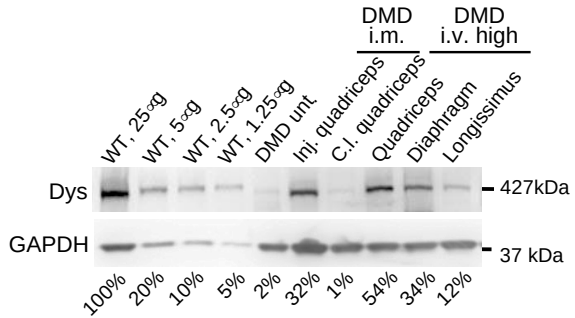


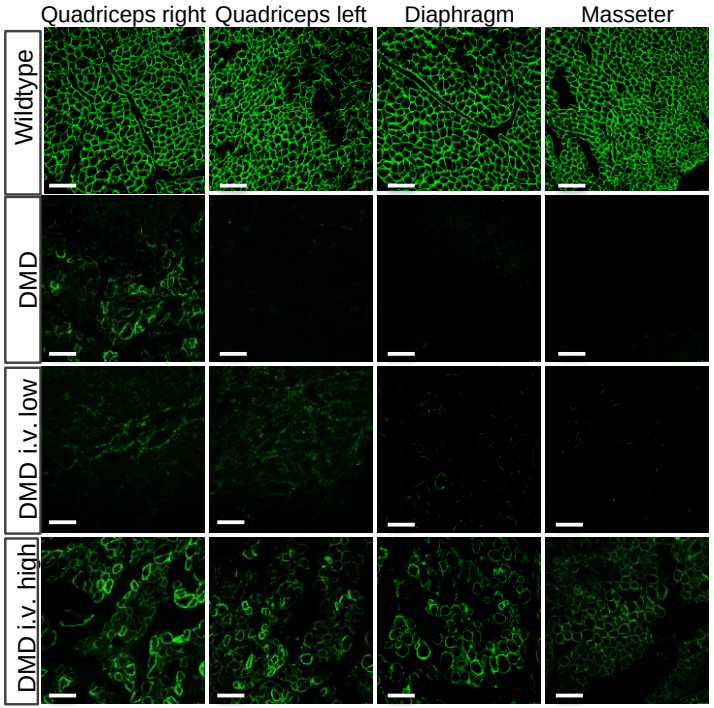




DMD pig (Δ52) treated with AAV-Cas9-gE51

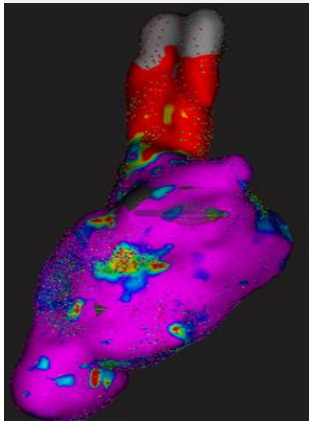




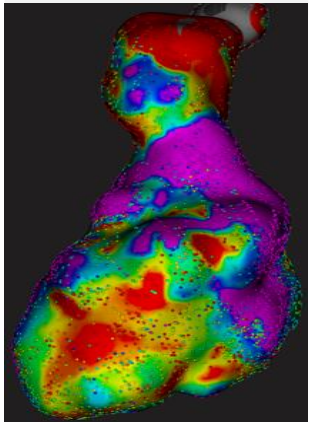


High resolution electrophysiologic analysis of DMD and BMD pigs

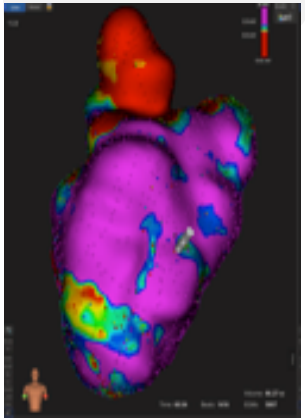
Wildtype



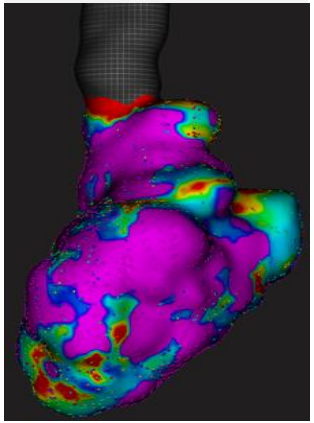
DMD untreated



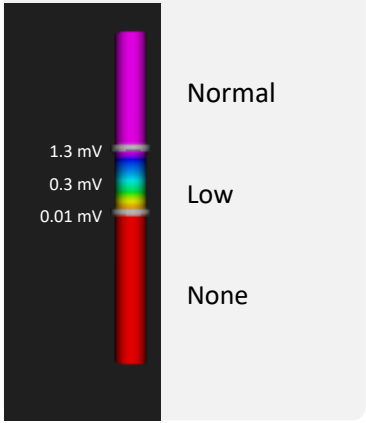
BMD



DMD i.v. treated

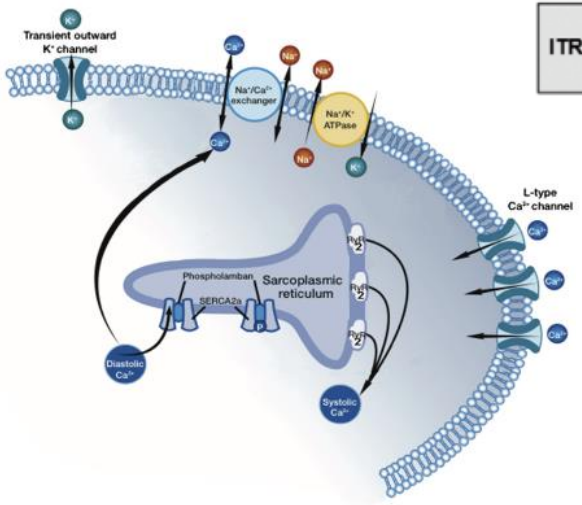


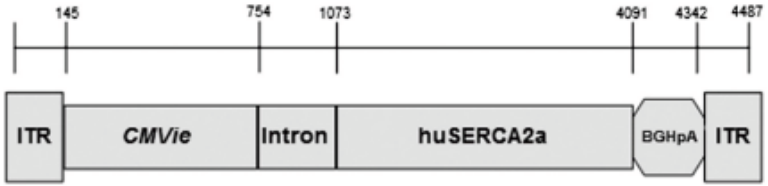
Action potential amplitude

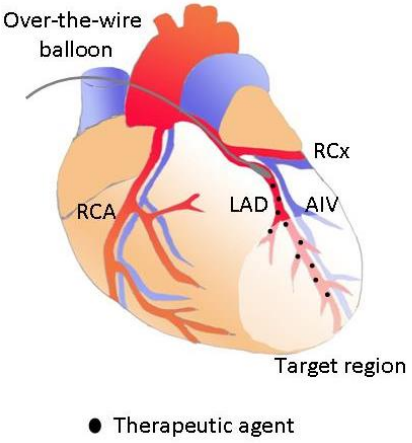


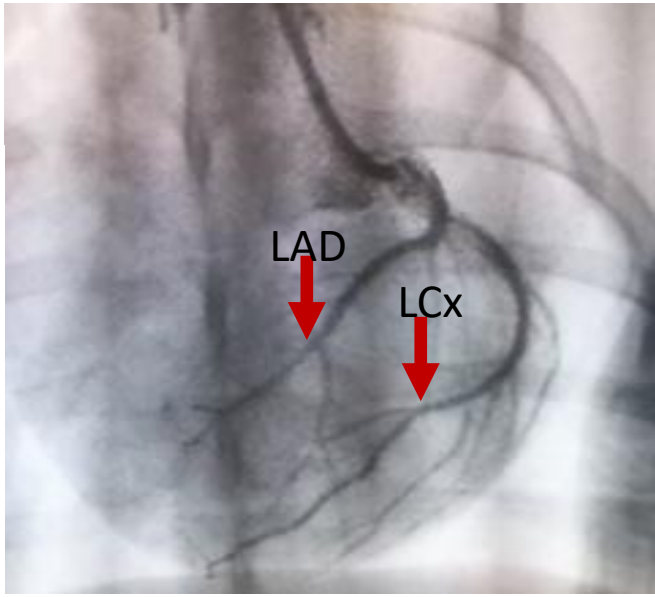
Moretti et al., Nat Med 2020

AAV1-Serca as heart failure gene therapy





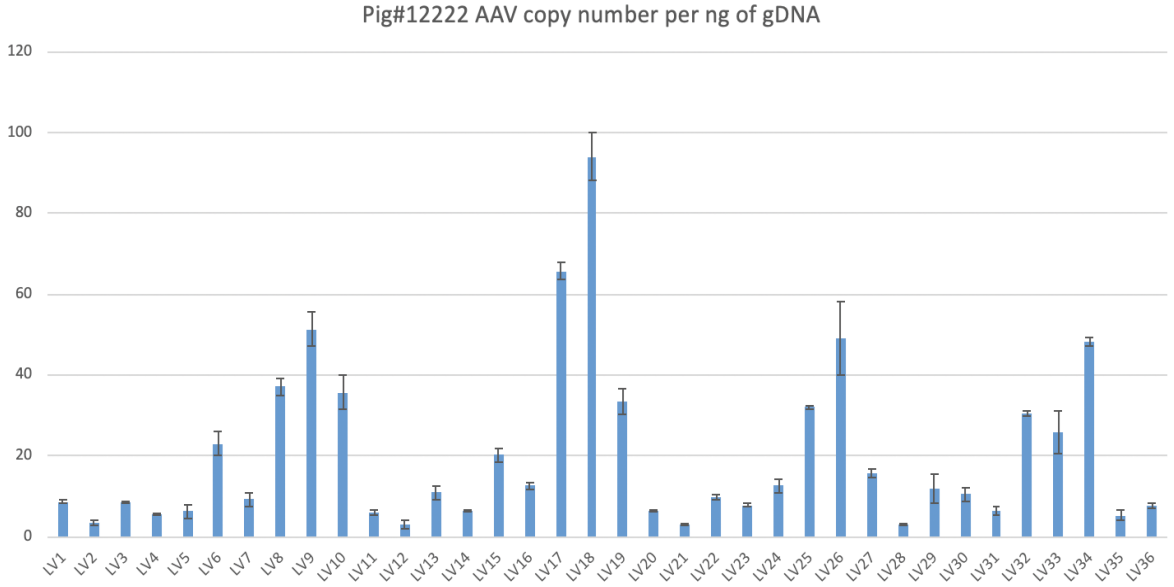


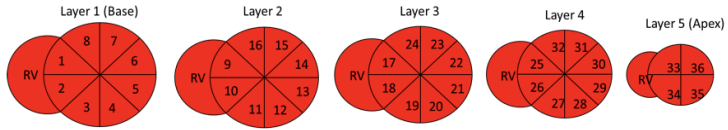


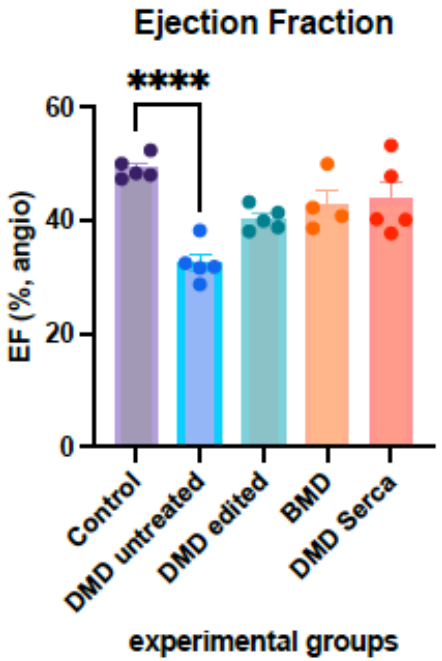
Greenberg et al.,
JACC Heart Failure 2014

Hinkel & Kupatt, Cardiovasc Res 2012

Serca2a Expression and function in pig hearts

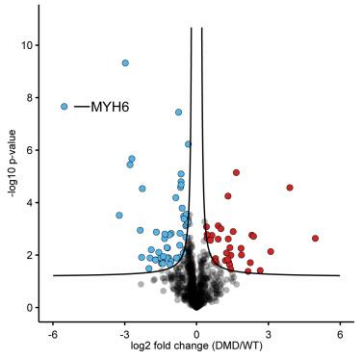




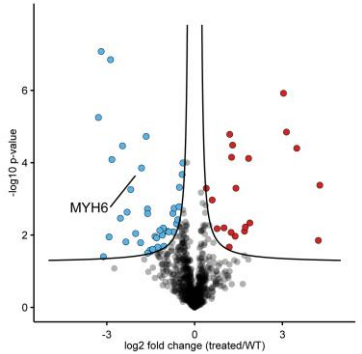


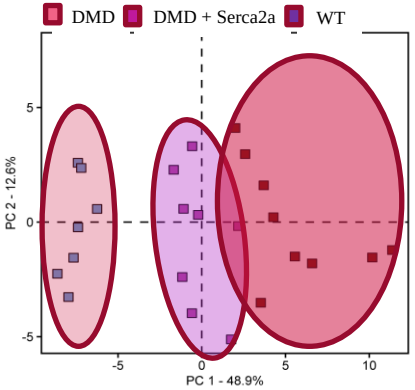
Proteomic analysis of DMD + Serca2a

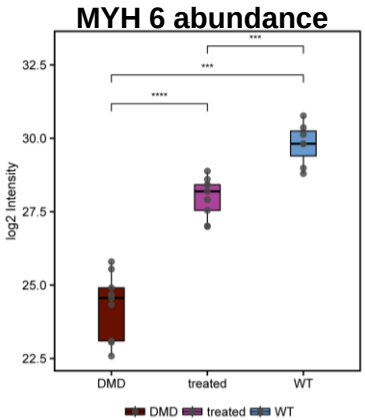
DMD untreated



DMD + AAV.Serca2a







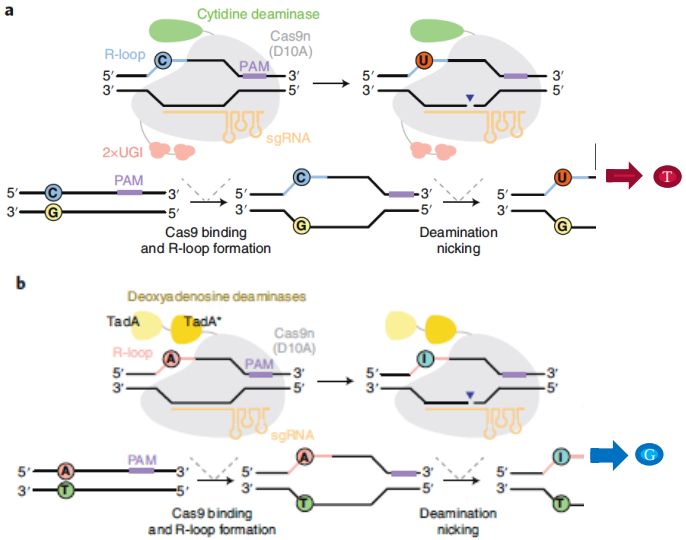
Blue significantly less abundant proteins
Red significantly higher abundant proteins

Summary

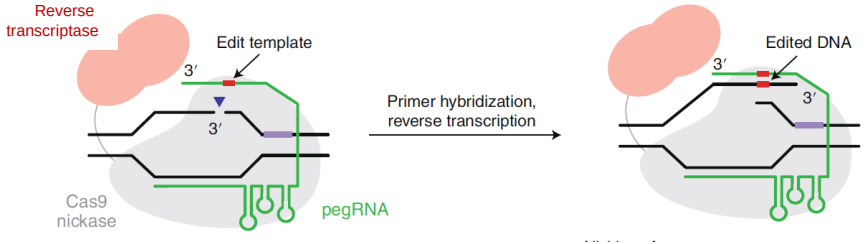
- DMD pigs suffer from heart failure and arrhythmogenic phenotype
- Gene editing can convert DMD to the milder phenotype of BMD, though full cardiac correction is a high aim
- AAV.Serca2a attenuates heart failure and related proteins.
  - Effects on arrhythmias less consolidated

CrispR Cas 2.0 – base and prime editing

CrispR 2.0 BASE EDITING

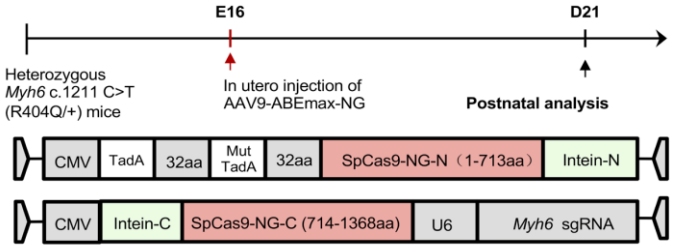


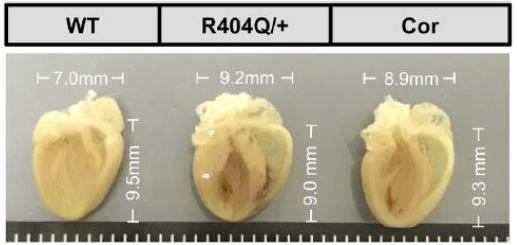
PRIME EDITING

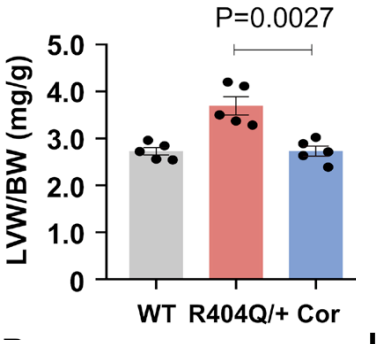


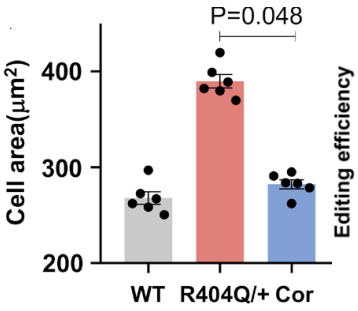
Anzalone et al., Nat Biotech 2020

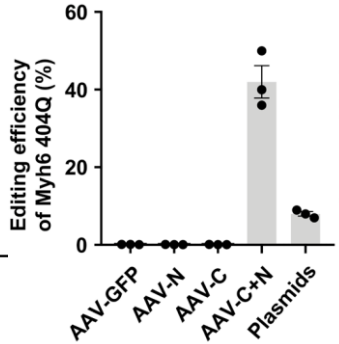
AAV-base-editing of R404Q in embryos

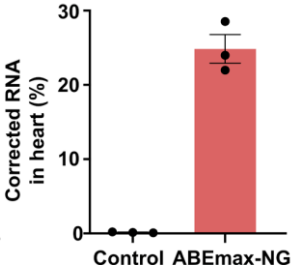






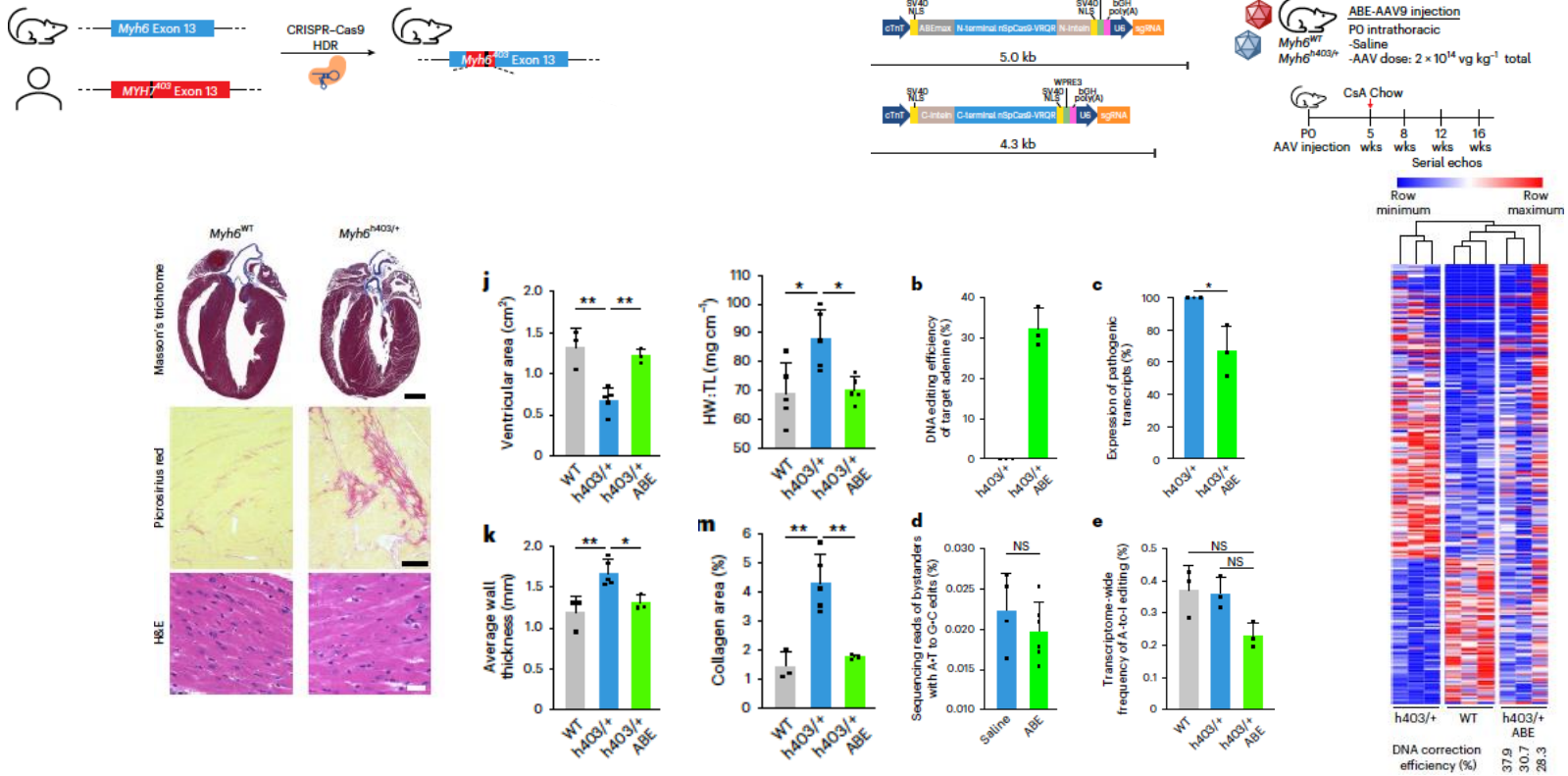






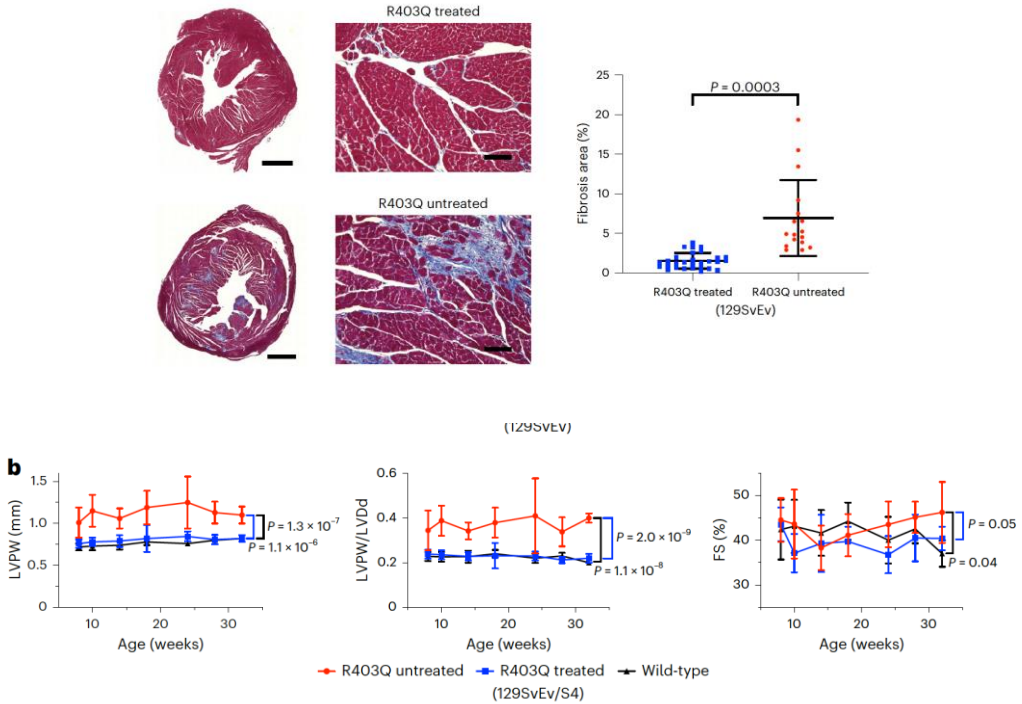
Ma et al., Circ Res 2021

Myosin Heavy Chain Myh6-R403Q base editing



Chai et al., Nat Med 2023

Myosin Heavy Chain 6 R403Q base editing (2)



Reichart et al., Nat Med 2023

Summary

- Genetic mutations inducing cardiomyopathies and skeletal muscle dysfunction are increasingly well understood
- This understanding forms the basis for novel therapeutics, either adjuvant or causal (gene editing)
- The presence of genetic cardiomyopathies is at times devastating, the future a lot brighter, and rapidly evolving.

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