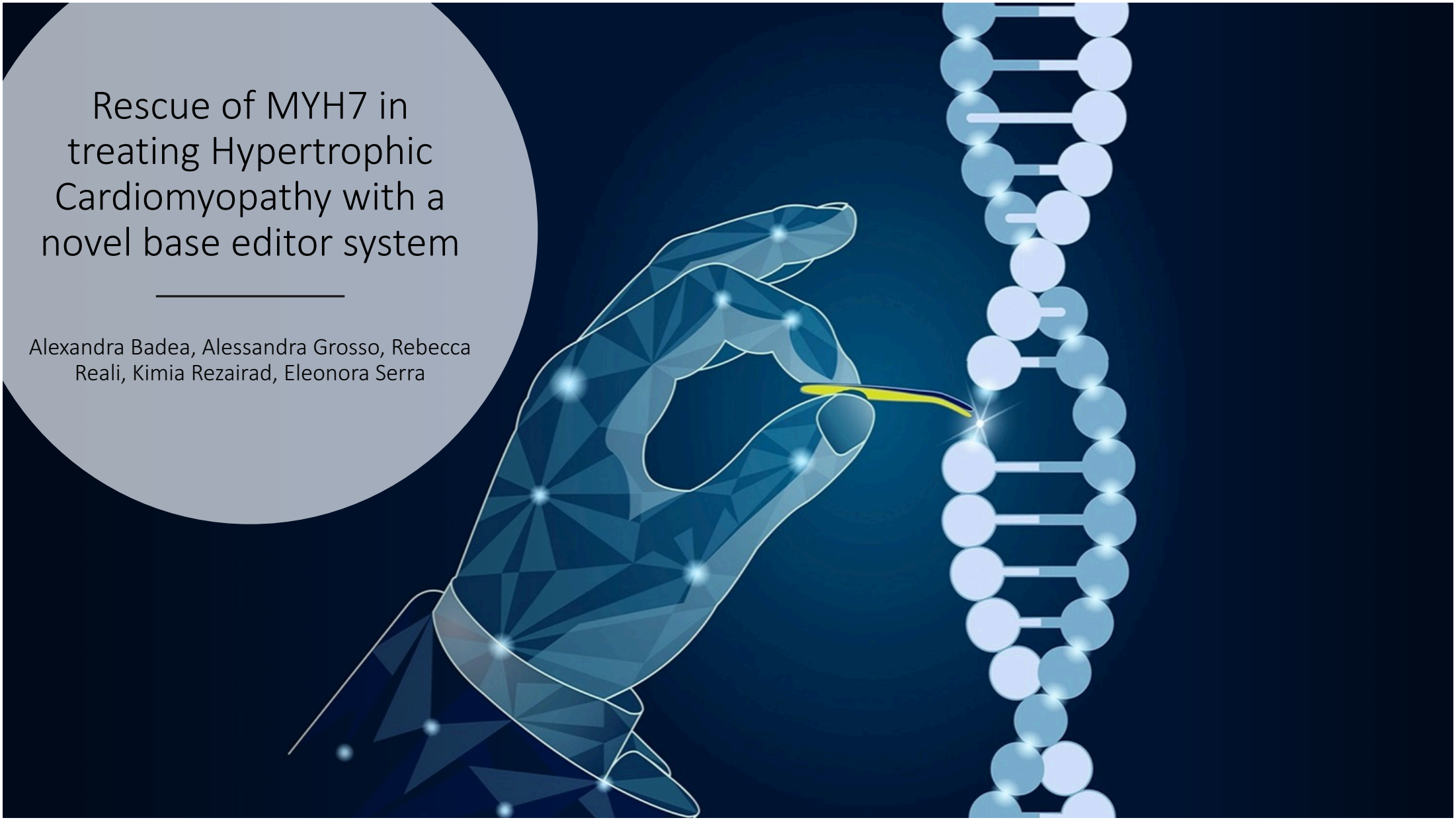
Rescue of MYH7 in treating Hypertrophic Cardiomyopathy with a novel base editor system

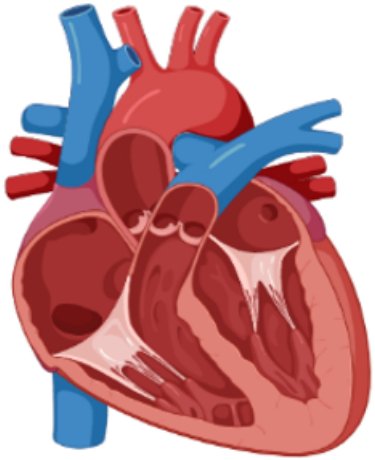
Alexandra Badea, Alessandra Grosso, Rebecca Reali, Kimia Rezairad, Eleonora Serra



What is Hypertrophic Cardiomyopathy?

- **Autosomal dominant disease** affecting 1/500 individuals
- Caused by mutations in sarcomeric genes, especially in **MYH7** and **MYBPC3**
- **Hallmarks**: hypertrophy of the left ventricle, cardiomyocytes disarray, fibrosis, hypercontractility and reduced compliance
- **Symptoms**: age-dependent and ranging from slight arrytthmias to sudden death

Normal heart



Hypertrophic Cardiomiopathy

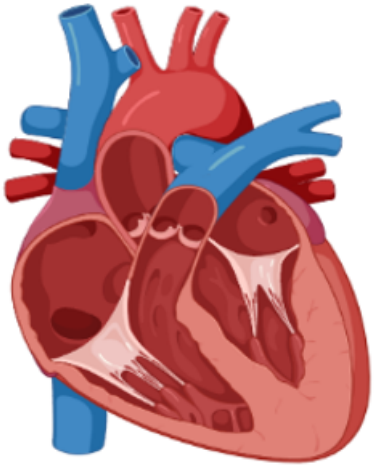
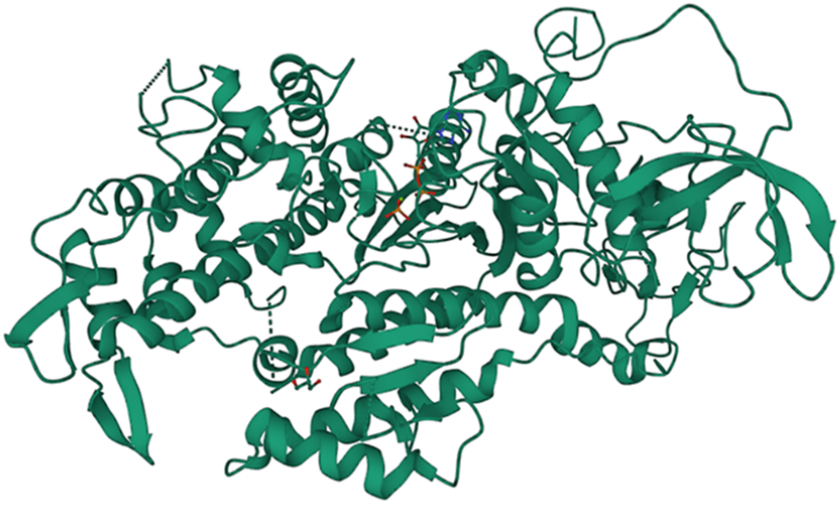
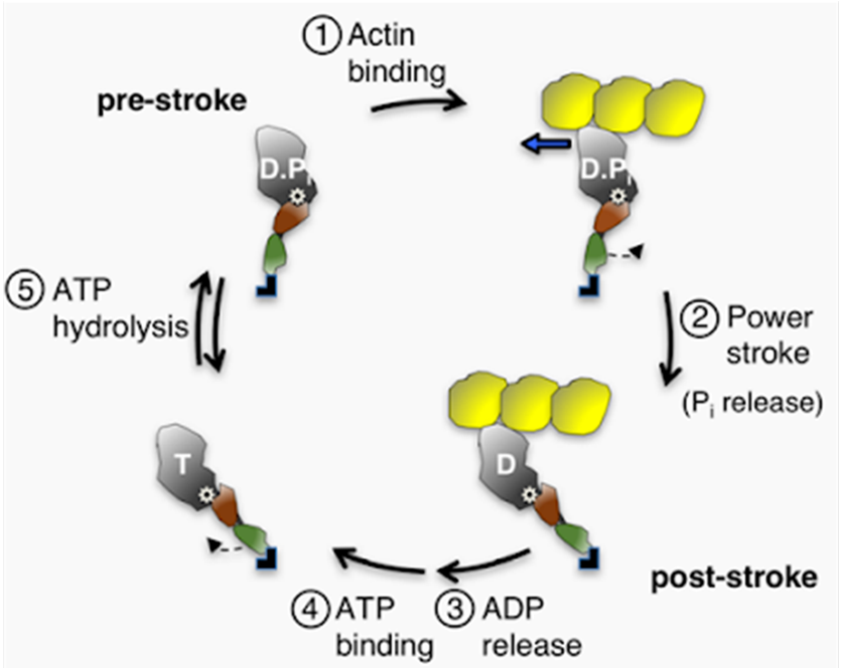


Image created with biorender

The cardiac muscle myosin motor domain

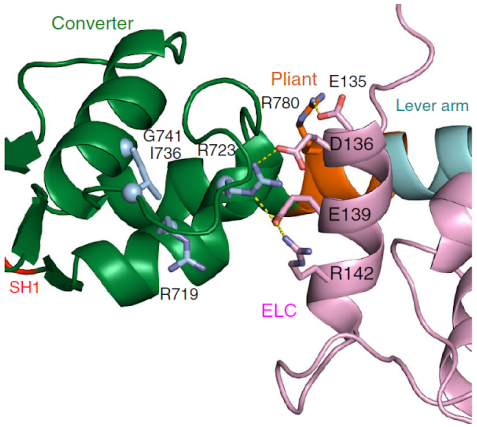


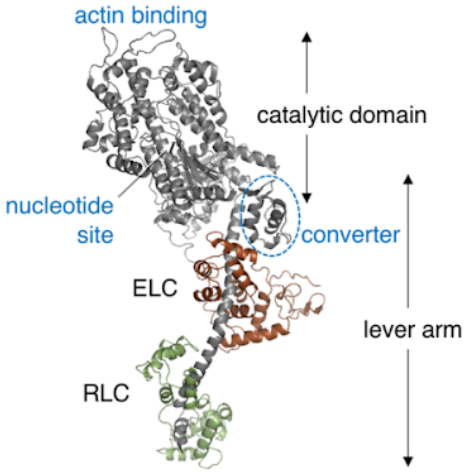
The ATPase cycle



Which are the effects of R723G mutation?

- Severe form of HCM
- Altered movement of the lever arm
- Muscle degeneration
- Fiber stiffness
- Removal of a charged residue crucial for the stabilization of the top loop conformation





Aim of the project

Correct the R723G point mutation directly on the genomic DNA to restore the wt phenotype.

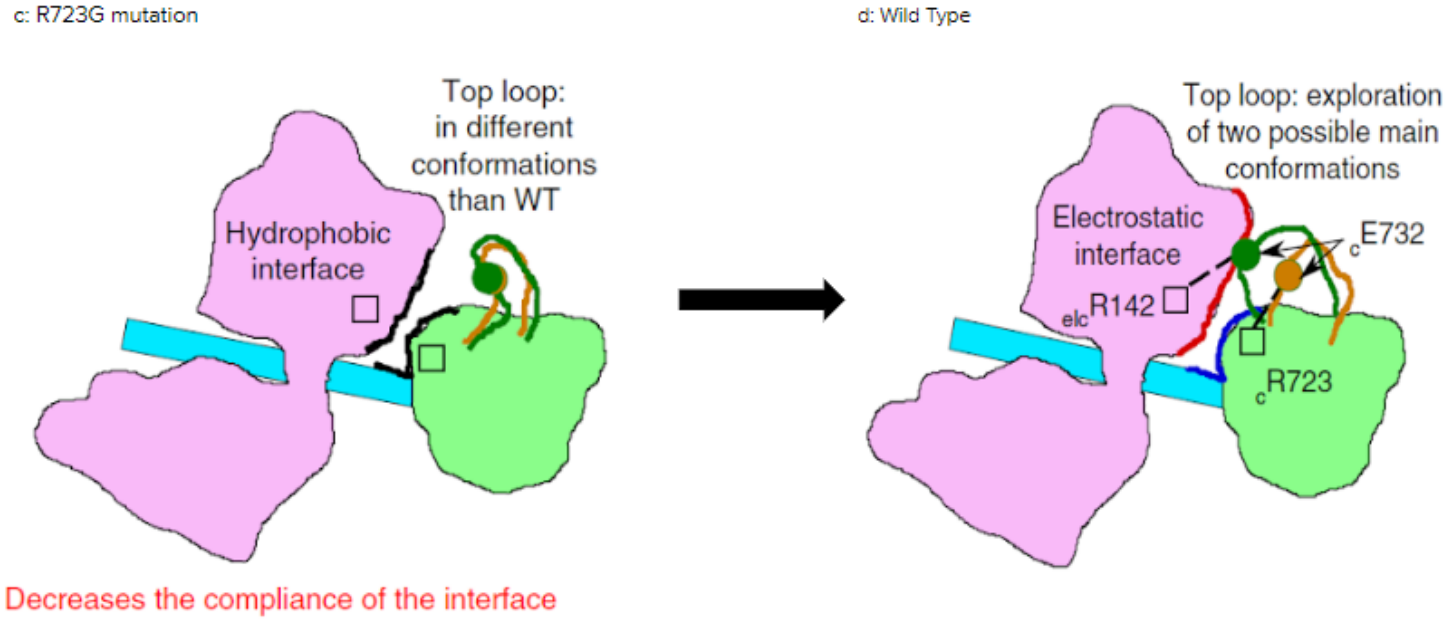
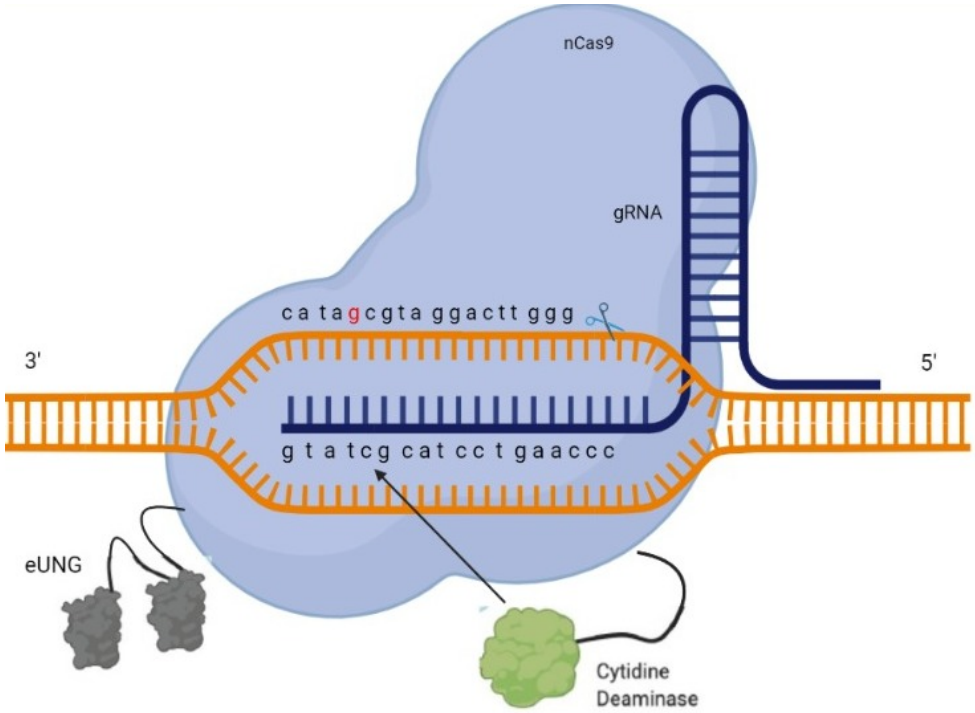


Fig. c,d: Robert-Paganin, J., Auguin, D. & Houdusse, A. Hypertrophic cardiomyopathy disease results from disparate impairments of cardiac myosin function and auto-inhibition. *Nat Commun* 9, 4019 (2018). https://doi.org/10.1038/s41467-018-06191-4

CGBE1

a recently developed base editor that allows C:G to G:C base transversions

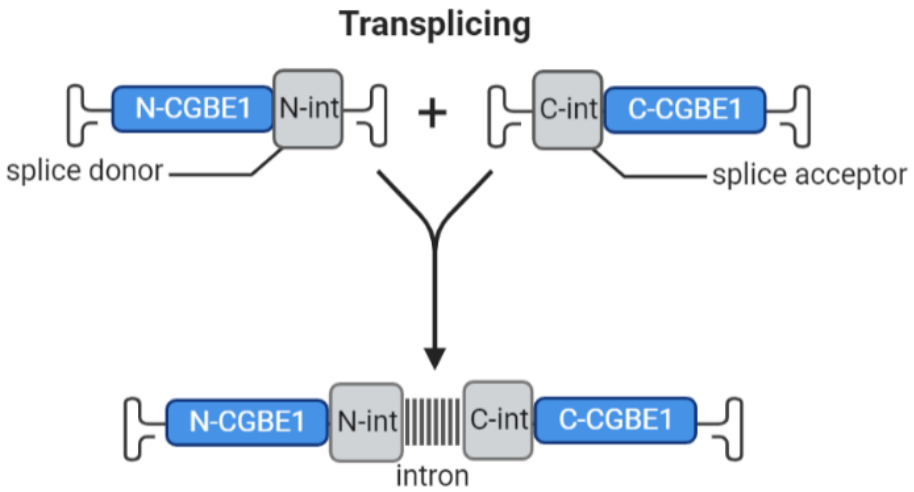


Components:

- RNA-guided Cas9 D10A nickase
- E. coli-derived uracil DNA N-glycosylase
- rat APOBEC1 cytidine deaminase variant (R33A)

How to fit a large base editor into the limited size of AAV?

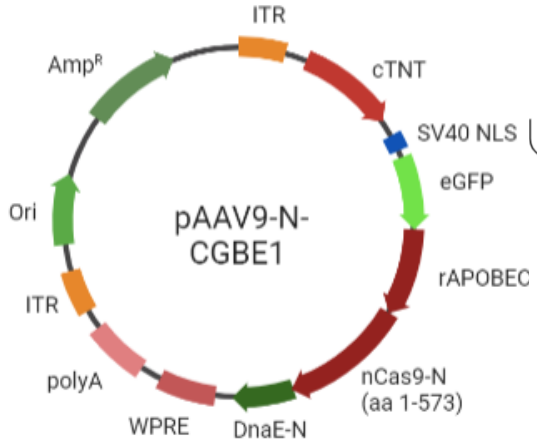
Development of
split-intein CGBE1 encoded into
dual-AAV particles to bypass
packaging size limit
of <5 kb of AAV.

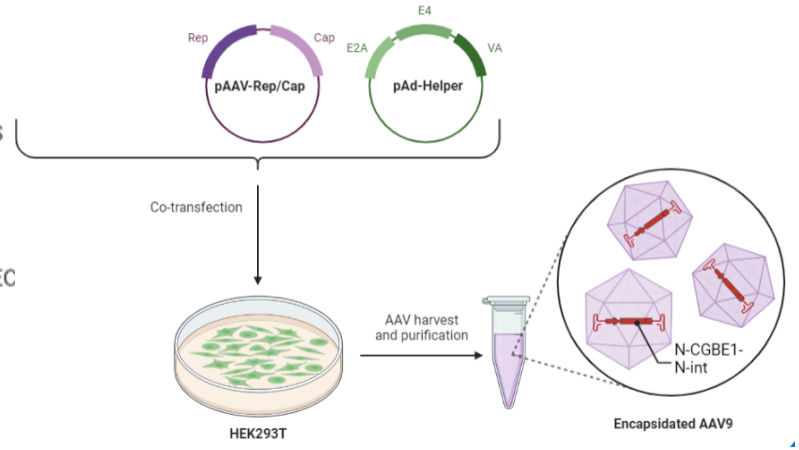


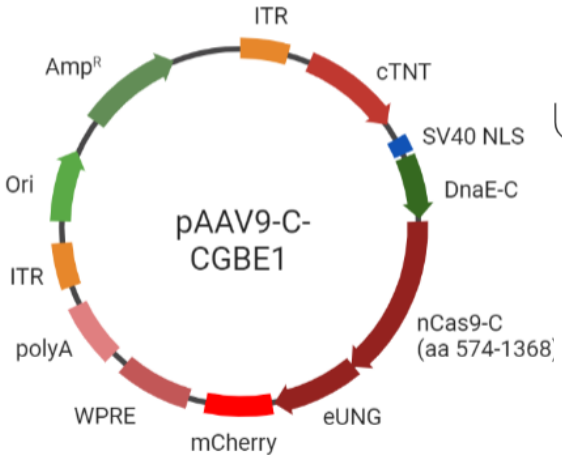
Split-intein CGBE1 dual-AAV9

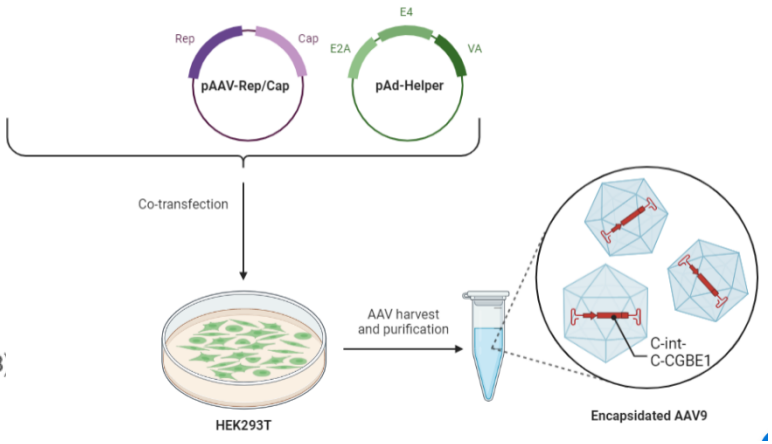
sgRNAs were designed using CHOPCHOP web tool:

5’ CCTGAGGAGGGAAGTGTCCAGAG 3’
5’ CAGGATGCGATACCTGAGGAGGG 3’
5’ TTCAGGATGCGATACCTGAGGAGGGAAGTGT 3’

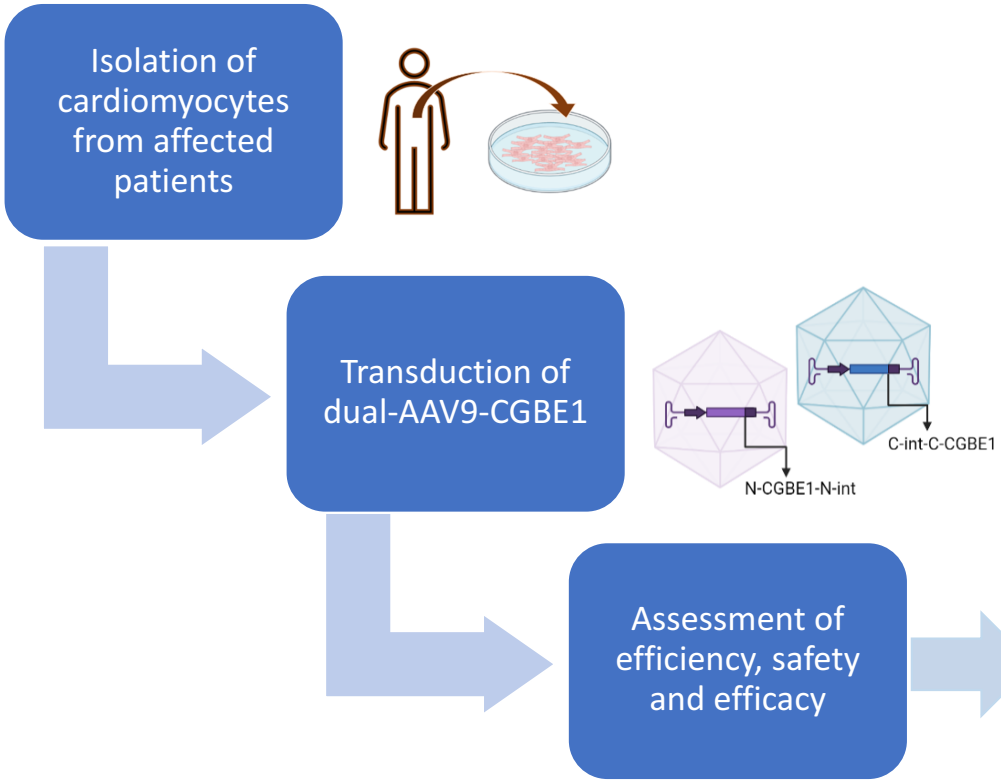








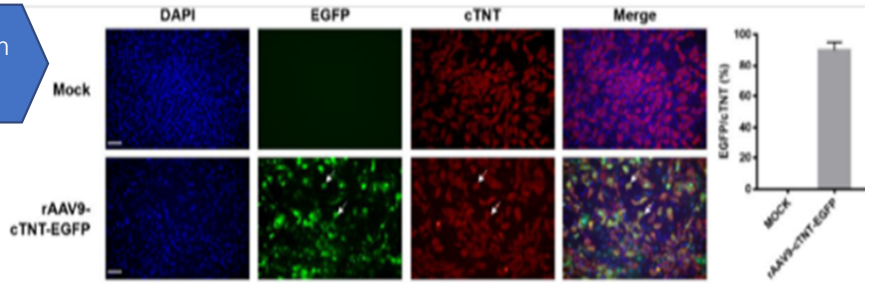
In vitro experimental plan



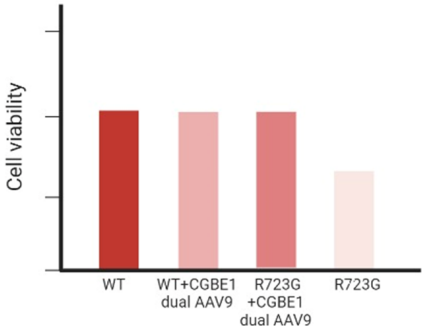
Transduction efficiency and cytotoxicity	• Immunofluorescence staining • Cell viability test
Base editing efficiency	• getPCR
DNA and RNA off-target and bystander edits	• Targeted DNA-seq at candidate off-target sites (identified by CIRCLE-seq) • Whole transcriptome RNA-seq
Expression levels	• Western blot
Phenotyping	• Hypertrophy analysis • High-content imaging • Mitochondrial respiration and content
Functional assays	• Calcium transient analysis and beat rate • Force measurements

In vitro results

Transduction efficiency

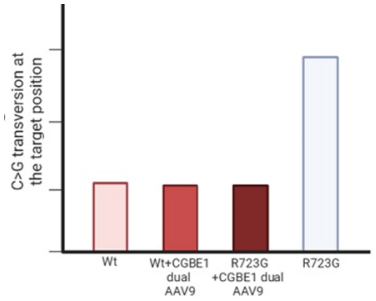


Cytotoxicity

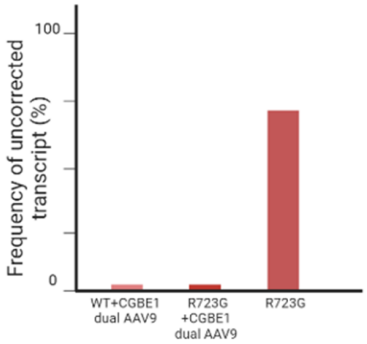


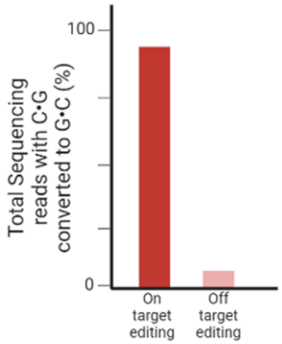
Sanger sequencing at the target codon (723)

Base editing efficiency by getPCR

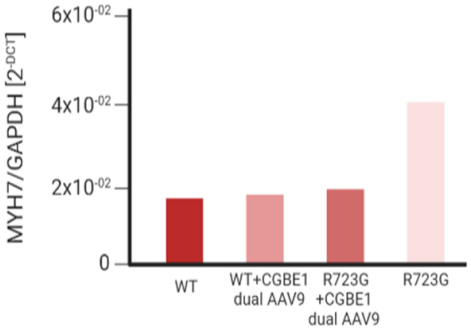


Analysis of off-target DNA and RNA editing



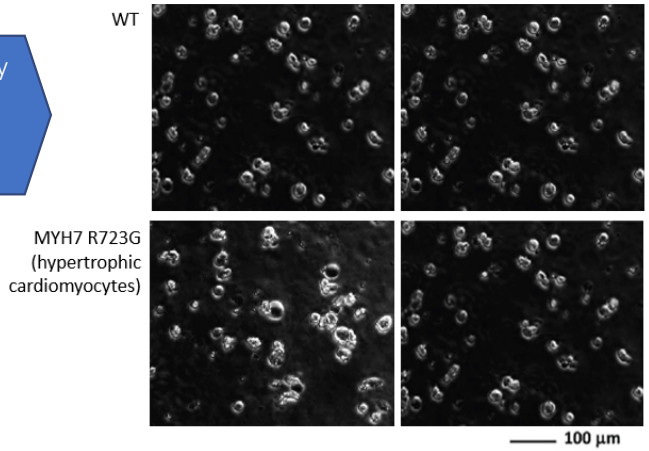


Relative expression of MYH7 mRNA per GAPDH by qPCR

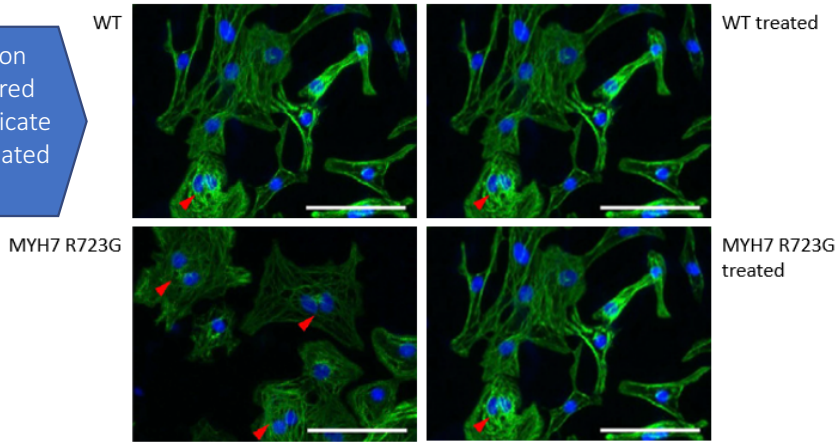


In vitro results

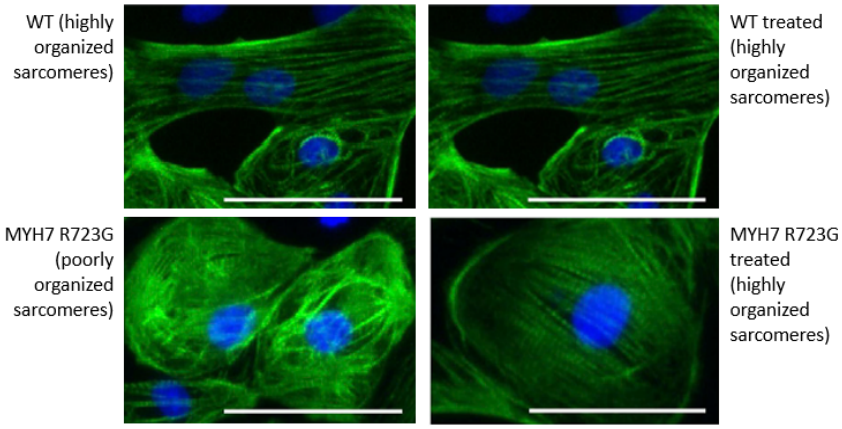
Hypertrophy analysis by bright-field imaging



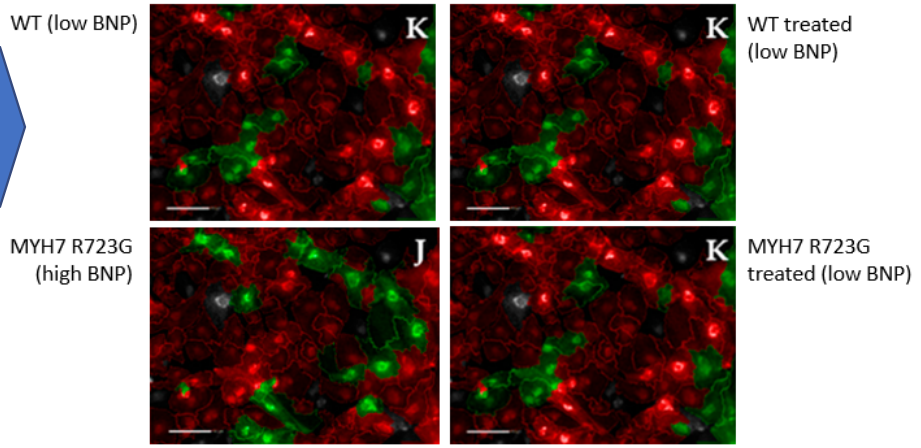
Nucleation analysis (red arrows indicate multinucleated cells)



Sarcomeric disarray

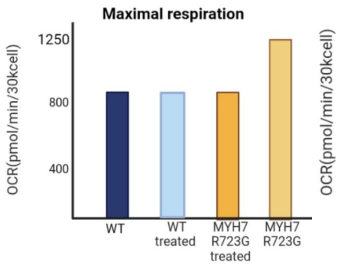


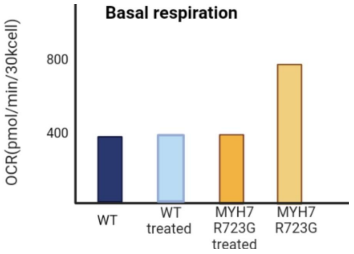
Hypertrophic marker BNP

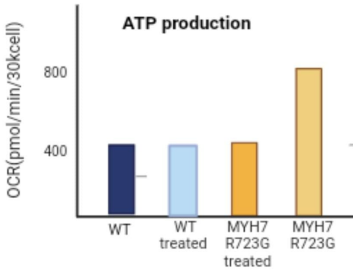


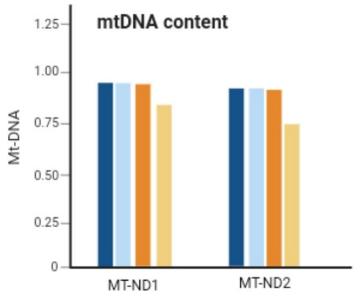
In vitro results

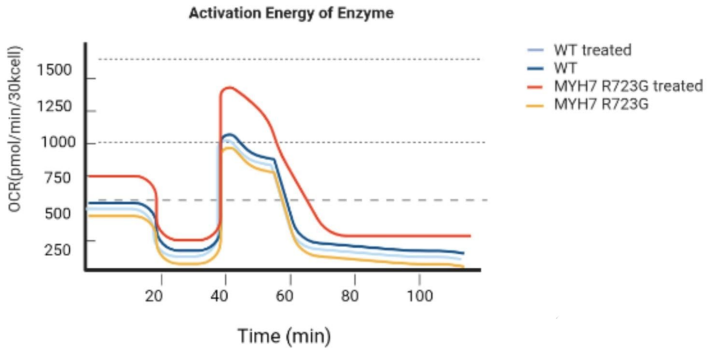
Mitochondrial respiration and content by Seahorse platform and qPCR



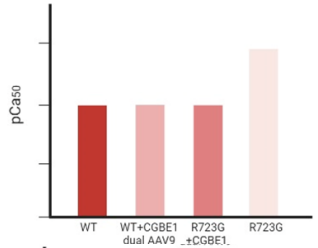


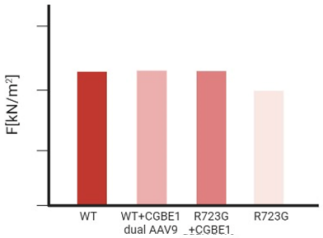


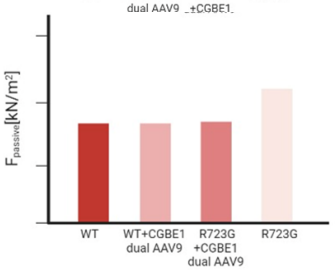


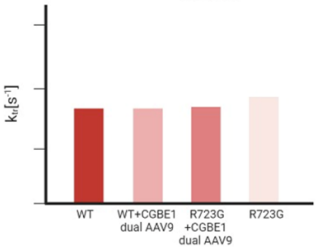


Force measurements

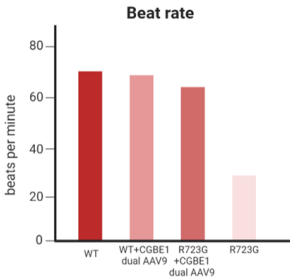


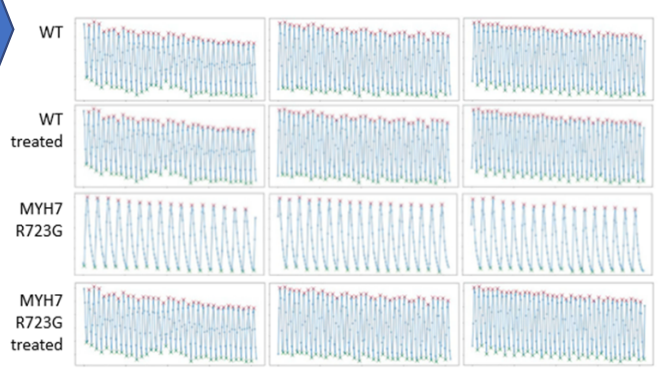






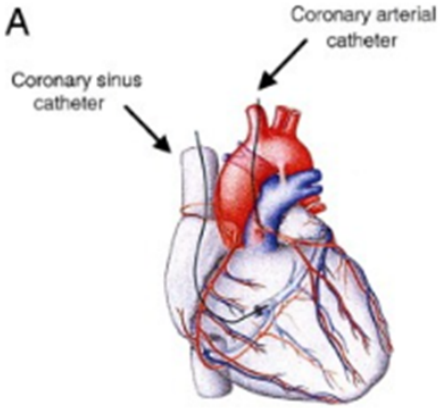
Calcium handling analysis

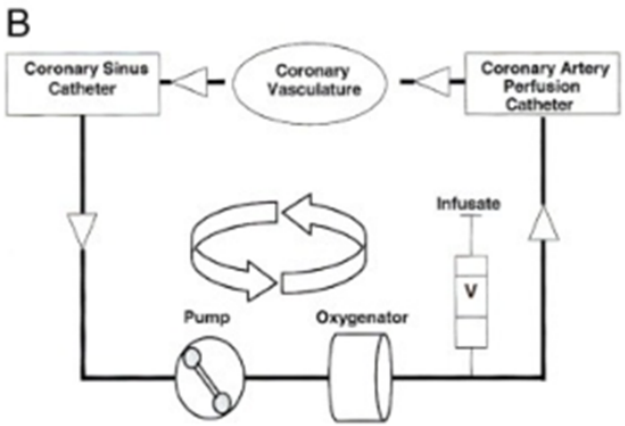




In vivo experimental plan

Knock-in of MYH7 R723G in pigs → Recirculating cardiac delivery of dual-AAV9 CGBE1 → Assessment of efficiency, safety and efficacy

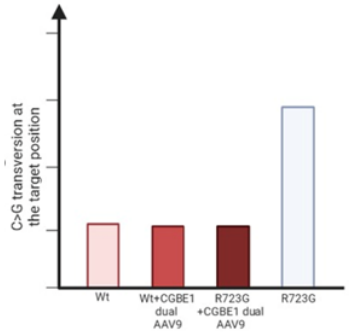




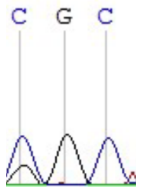
AAV distribution	• Immunohistochemistry
Base editing efficiency	• getPCR
DNA and RNA off-target and bystander edits	• Whole genome sequencing • RNA-seq
Expression levels	• Western blot
Morphological and functional assays	• Histopathology • Contractility assay • Ecocardiography • Electrophysiology

In vivo results

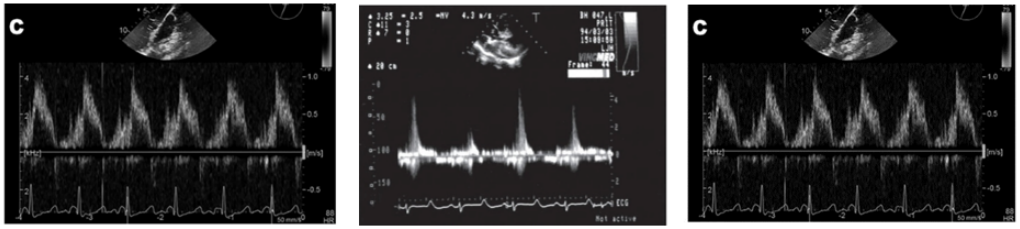
Base editing efficiency by getPCR



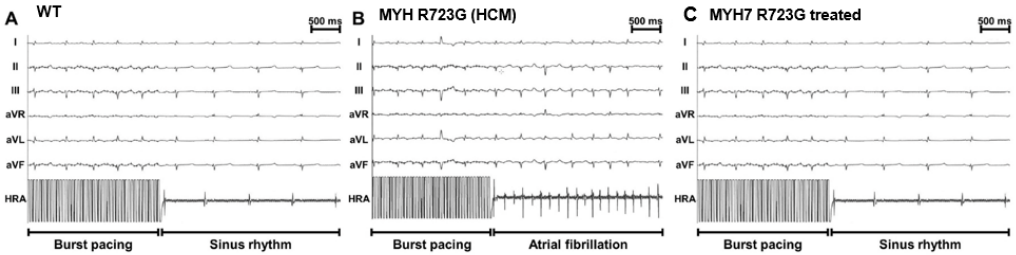
Sanger sequencing at the target codon (723)



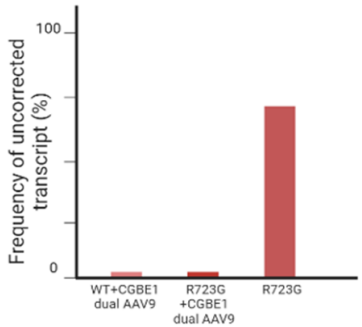
Ecocardiography

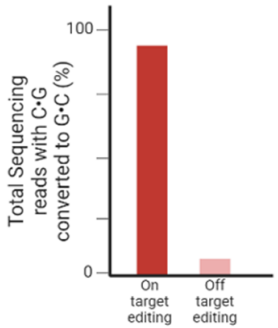


Electrophysiology

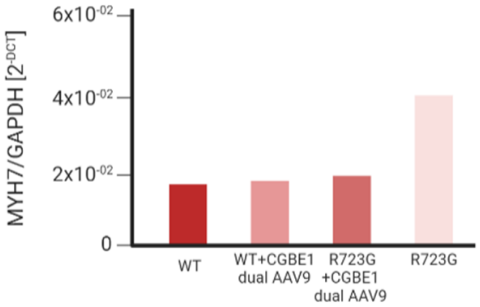


Analysis of off-target DNA and RNA editing



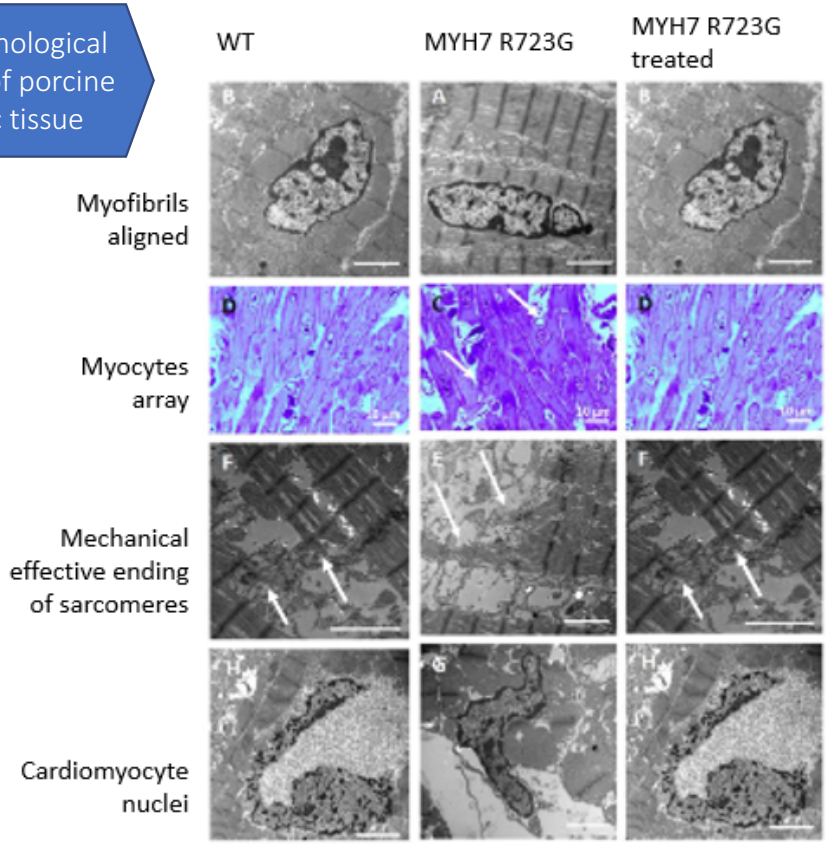


Relative expression of MYH7 mRNA per GAPDH by qPCR

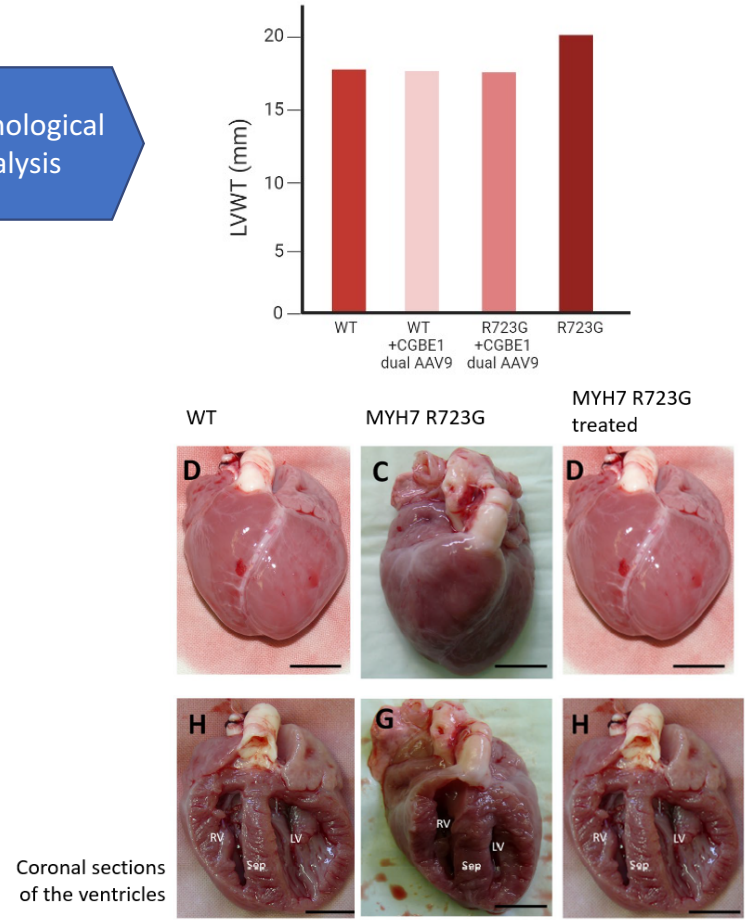


In vivo results

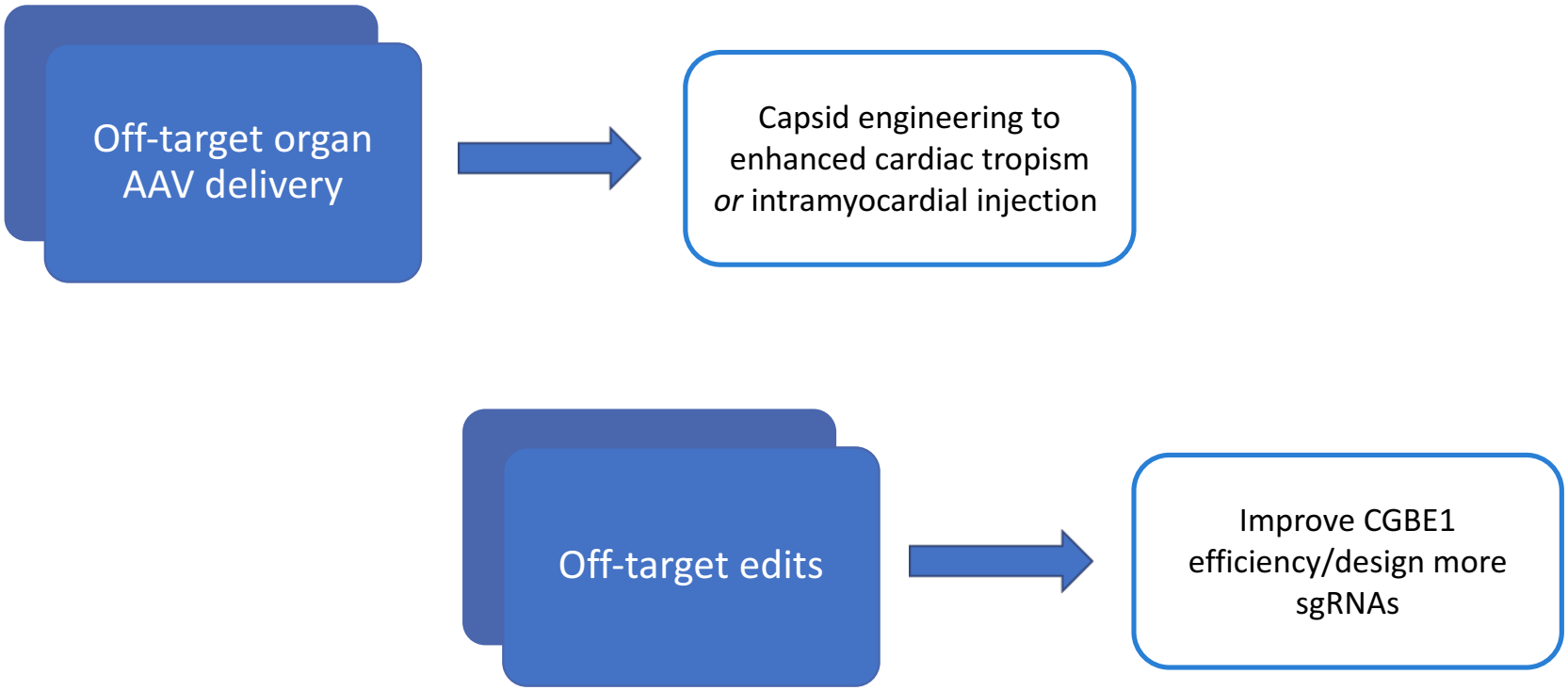
Histopathological analysis of porcine cardiac tissue



Morphological analysis



Pitfalls and solutions



Budget

REF	Headings	Costs ($)	
	1. eligible direct costs		
A	Personnel: Researcher salary	35000$ per year; x5: 175,000$	
B	Equipment:	- WT Swine: 550$ per animal (28 days of age) x5 = 2,750$ - Genetically modified Swine: 2,250.00$ per animal (28 days of age) x5 = 11,250 $	
	2. eligible indirect costs		
C	Supplier Tooling:	GET-PCR Kit: 130$	Cell Viability: 300$
		Western Blot: 2,000$	Echocardiogram: 1,600$
		AAV9 Vectors: 4,000 $ x 2 = 4,000$	Electrophysiology: 4,000$
		Immunofluorescence Staining Kit: 90$	RNASeq Oxford Nanopore:: 300$
		Immunohistochemistry: 290$	Cell Contraction Assay: 475$
		Illumina Whole genome Seq: 200$	Targeted deep DNA Seq: 350$
		Sanger Seq Kit: 600$	
	Total: 1+2 = 189,000$ + 14,335$ = 203,335$ per year		


Thank you for your attention

Bibliography

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