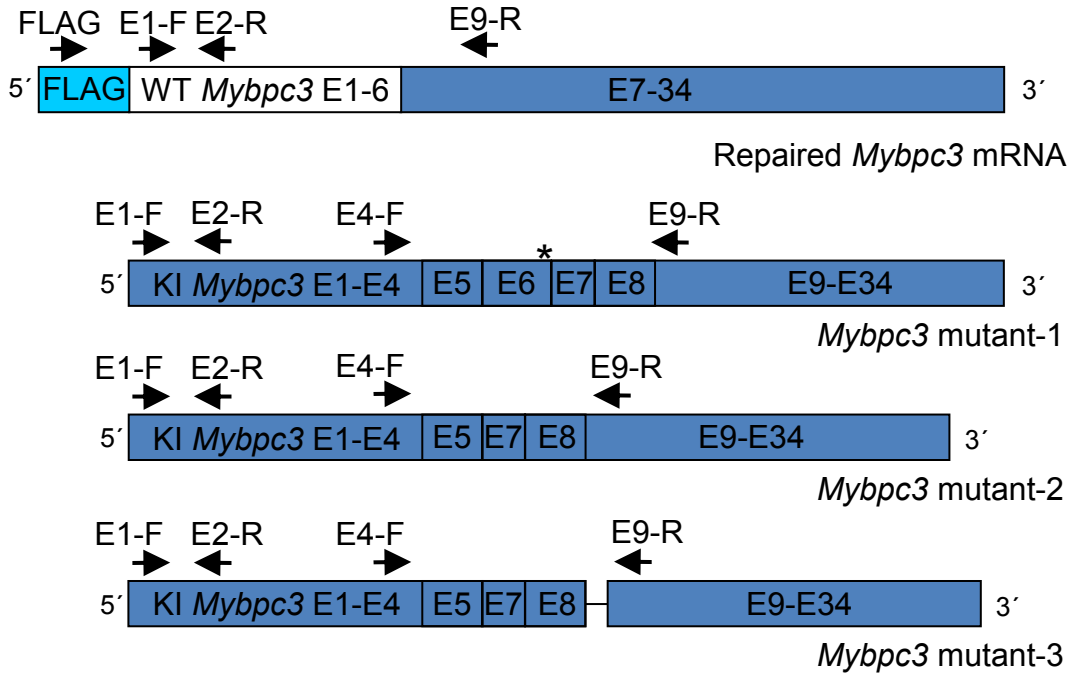

	FLAG/E9-R	E1-F/E9-R	E1-F/E2-R	E4F/E9-R
repaired *Mybpc3*	921 bp	896 bp	242 bp	415 bp
Mutant-1	na	896 bp	242 bp	415 bp
Mutant-2	na	778 bp	242 bp	297 bp
Mutant-3	na	824 bp	242 bp	334 bp

Figure S1 Schematic representations of the repaired and mutant *Mybpc3* mRNAs. *Mybpc3*-targeted knock-in (KI) mice carry a homozygous G>A transition on the last nucleotide of exon 6. This mutation gives rise to 3 different mutant mRNAs. Mutant-1 mRNA (missense) contains the G>A transition (*). Mutant-2 mRNA (nonsense) results from the skipping of exon 6. Mutant-3 mRNA (deletion/insertion) results from the skipping of exon 6 and retains a part of intron 8. The arrows indicate the position of primers used. The predicted size of PCR products is given in the table below. Abbreviations: E, exon; F, forward; R, reverse; na, not amplified.

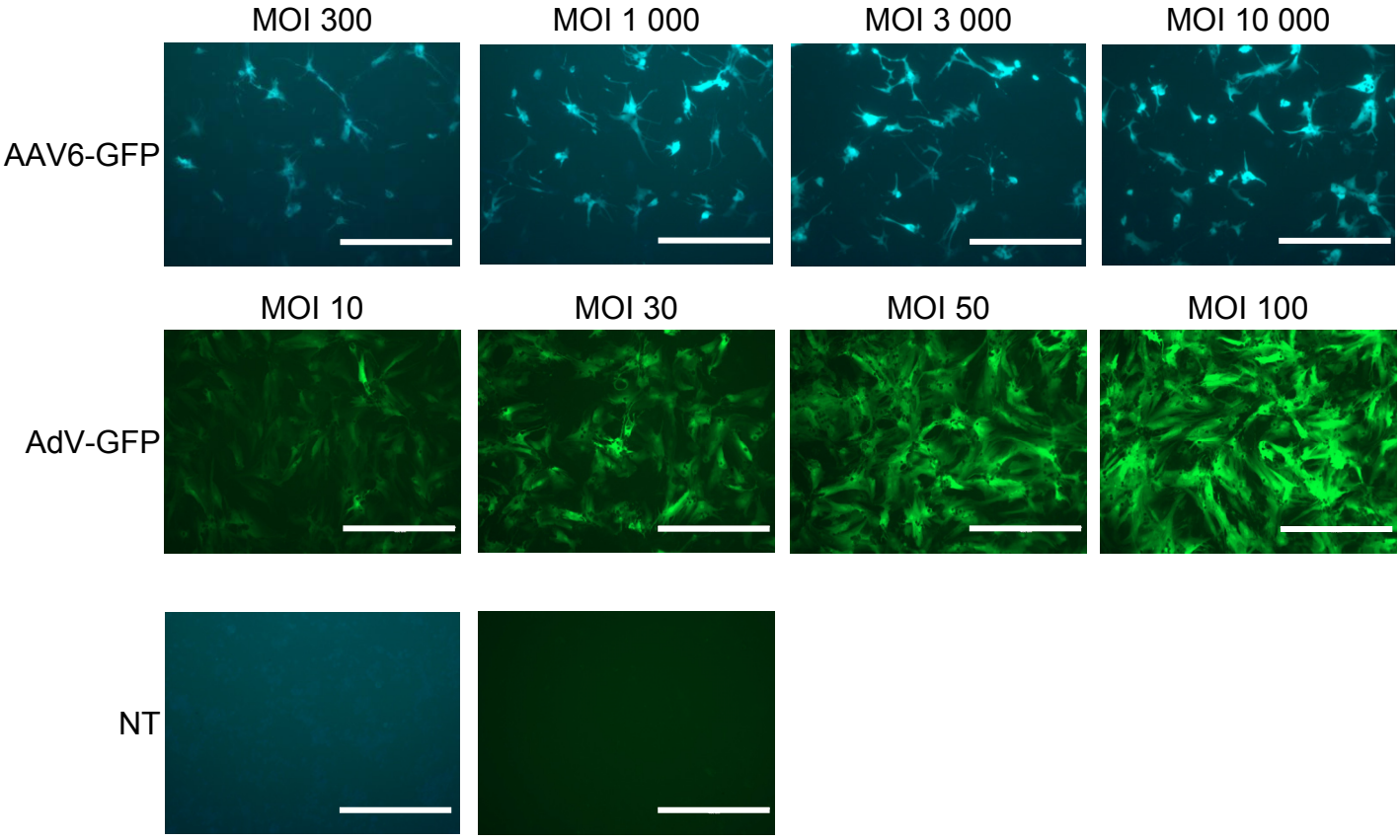

Figure S2 Efficiency of AAV6- and AdV-mediated transduction in *Mybpc3*-targeted knock-in cardiac myocytes. Cardiac myocytes were isolated from KI neonatal mice and transduced with different MOIs of adeno-associated virus serotype 6 (AAV6) or adenovirus (AdV), both encoding GFP under the control of human cardiac troponin T promoter (*TNNT2*). GFP expression was evaluated by epifluorescence microscopy with a 10x objective 4 days post-transduction and compared to non-transduced (NT) cardiac myocytes. These data show that about 80% of cells were green after AAV6-mediated transduction with a MOI of 3 000 and 100% after AdV-mediated transduction with a MOI of 100. Scale bar 400 µm.

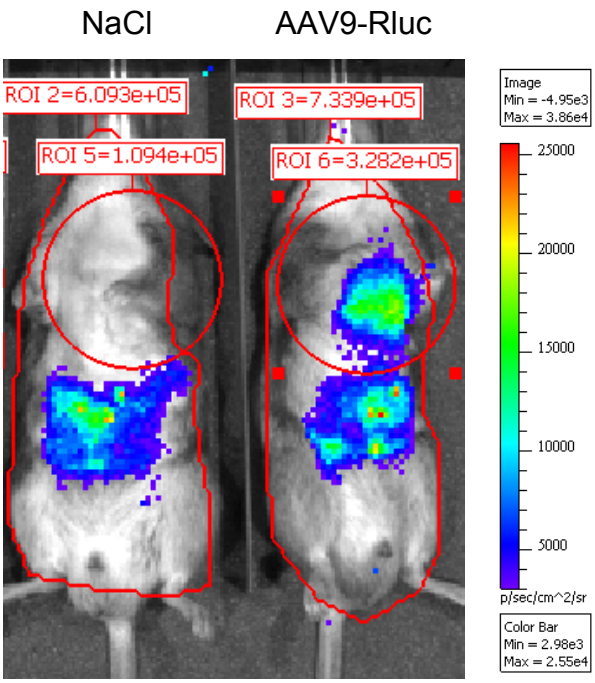

Figure S3 *In vivo* bioluminescence imaging. *In vivo* bioluminescence imaging was performed 28 days after *i.p.* injection of 2.5 mg/kg body weight luciferase substrate (coelenterazine) in mice. Light emission was recorded specifically in the heart of the mouse that received AAV9-RLuc. The positive signal at the site of *i.p.* injection in both NaCl- and AAV9-RLuc-injected mice corresponded to autofluorescence of the substrate. Images were taken 28 days after injection. Abbreviations: *i.p.*, intraperitoneal; ROI, region of interest.

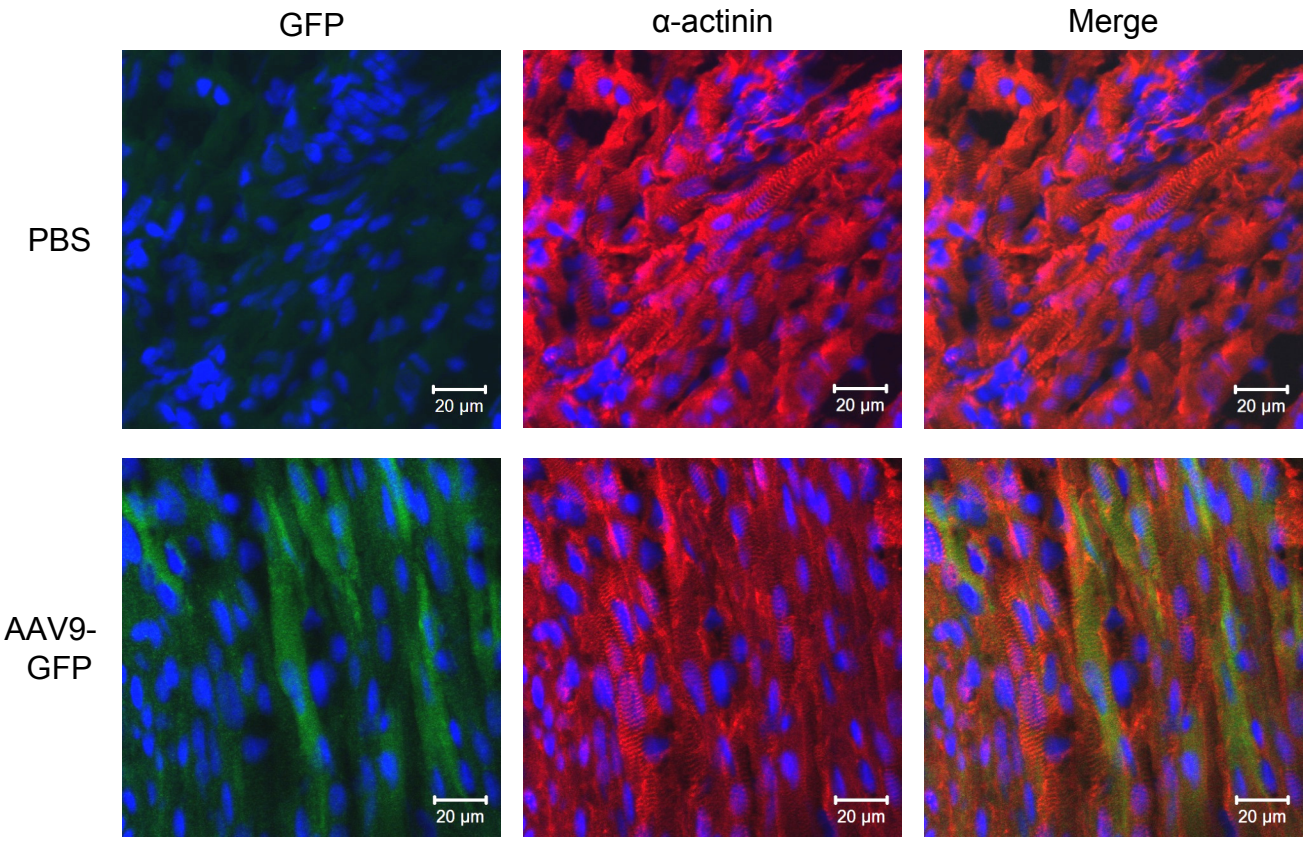

Figure S4 Efficiency of transduction after systemic administration of AAV9-GFP in a *Mybpc3*-targeted knock-in mouse. AAV9-GFP ($1.5x10^{14}$ vg/kg body weight) was administered into the temporal vein of 1-day-old KI mouse for 7 days. Another KI mouse received the same volume of PBS and was used as control. The hearts were extracted and, after rinsing in KCl, embedded in Tissue-Tek®. Cryosection of 10 µm thickness were cut and stained with primary antibodies directed against GFP (1:200, Santa Cruz) and α-actinin (1:800, Sigma) and with the secondary antibodies anti-rabbit Alexa 488 (1:600, Molecular Probes) and anti-mouse Alexa Fluor 546 (1:600, Molecular Probes). Nuclei were stained with DRAQ5™ (1:800). After embedding in Mowiol, immunofluorescence analysis was performed by confocal microscopy with a 40x-oil objective.

After 7 days of transduction GFP-positive cardiac myocytes were detected in the entire section of the AAV9-CMV-GFP-injected mouse but not in the PBS-injected mouse. This suggests very high cardiac transduction efficiency with, and therefore confirms the high cardiac tropism of AAV9.

We thank V. Behrens-Gawlik (Pharnacology, UKE, hamburg) for taking the images.

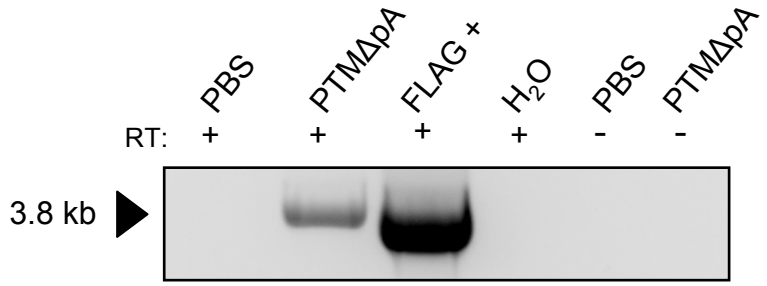

Figure S5 Detection of full-length repaired *Mybpc3* mRNA. The cardiac cDNA obtained from a mouse transduced with AAV9-PTMΔpA or PBS for 7 weeks was amplified with FLAG and *Mybpc3* exon 33 primers. A fragment (3.8 kb) was detected only in the AAV9-PTMΔpA-injected mouse and in the positive control (FLAG +), but not in PBS-injected mouse or in the amplifications from samples without reverse transcriptase (-).

Binding domain (BD)	Sequence (5´ to 3´)	Size (nt)
BD1	cagcccaagctgatcctctagagtcgaccacagctagtcaccctctctagttgggaga tagagacaagcccaagggaccaggacccatgaagaccacaagacca	104
BD2	ggaacccttaatataacttcgtataatgtatgctatacgaagttattaggtccctcgacct gcagcccaagctgatcctctagagtcgaccacagctagtcaccctctctagttgggag a	120
BD3	tgaggcagtttctcatctcagtctccacagtgggctgcctcccactcacctcccagttca cattcaaactgtgtgaggcagggacacagaaacagcatcccacccttgacttgctcc cacgattctcctgaaagcatgcatttcgttcacacctcg	156
Reverse BD1	tggtcttgtggtcttcatgggtcctggtcccttgggcttgtctctatctcccaactagagag ggtgactagctgtggtcgactctagaggatcagcttgggctg	104
Reverse BD2	tctcccaactagagagggtgactagctgtggtcgactctagaggatcagcttgggctg caggtcgagggacctaataacttcgtatagcatacattatacgaagttatattaagggtt cc	120
Reverse BD3	cgaggtgtgaacgaaatgcatgctttcaggagaatcgtgggagcaagtcaagggtg ggatgctgtttctgtgtccctgcctcacacagtttgaatgtgaactgggaggtgagtggg aggcagcccactgtggagactgagatgagaaactgcctca	156

Supplementary Table S1 Sequences of the binding domains. Sequences of the different binding domains (BD1 to BD3) that are complementary to intron 6 of KI *Mybpc3* and of the reverse binding domains (reverse BD1 to BD3), which do not base-pair with intron 6. Abbreviation: nt, nucleotide.

Primer	Sequence (5´ to 3´)
FLAG	GGATTACAAGGATGACGACGA
E1-F	CCTGGTGTGACTGTTCTCAA
E2-R	GTCATCAGGGCTCGCATC
E4-F	TCTTTCTGATGCGACCACAG
E9-R	TCCAGAGTCCCAGCATCTTC
E33-R	TGCGGAAGCGAGCATCTT
Luciferase F	GTAACGCTGCCTCCAGCTAC
Luciferase R	CCAAGCGGTGAGGTACTTGT
Myh6 F	CTCAAGCTCATGGCTACACTCTTCTC
Myh6 R	AGAGCAGACACTGTTTGGAAGGA
Gapdh F	ATTCAACGGCACAGTCAAG
Gapdh R	TGGCTCCACCCTTCAAGT
PTM F	TTCGACCTCGAGATGGATTACAAGGATGACGACGATAAGCCTG GTGTGACTGTTCTCAA
PTM R	TTCGACGGATCCAGGCCAACCCATGGAAAGAAAGAGCTGTAC TCACCATGGACAGTGAGGTTGAAGTTA
BD F	TTCGACGGATCCTCTCCCAACTAGAGAGGGTGA
BD R	TTCGACGCGGCCGCGGAACCCTTAATATAACTTCGTATAATG
BD-R F	TTCGACGGATCCGGAACCCTTAATATAACTTCGTATAATG
BD-R R	TTCGACGCGGCCGCTCTCCCAACTAGAGAGGGTGA
TNNT2 F	GCATCAGTTCAAGTGGAGCA
TNNT2 R	GGGACAAGGCTACAGGAACA

Supplementary Table S2 Sequences of the PCR primers used in the present study. Sequences are given from 5´ to 3´.

Age at Echo		**LVM/BW (mg/g)**	**FAS (%)**
11-wk-old	NaCl	6.22	31.52
11-wk-old	PTM Δ pA	6.01	20.87
11-wk-old	RLuc	5.29	27.90
7-wk-old	PBS	5.37	28.07
7-wk-old	PTM Δ pA	6.55	22.22

Supplementary Table S3 Echocardiographic analysis. Echocardiographic analysis was performed 4 or 7 weeks after AAV9- and NaCl- or PBS- injections in 7-week-old or newborn mice, respectively. Left ventricular mass to body weight ratio (LVM/BW) and fractional area shortening (FAS) are listed.