Supplemental Material

CRISPR-Cas9 base editing of pathogenic CaMKIIδ improves cardiac function in a humanized mouse model

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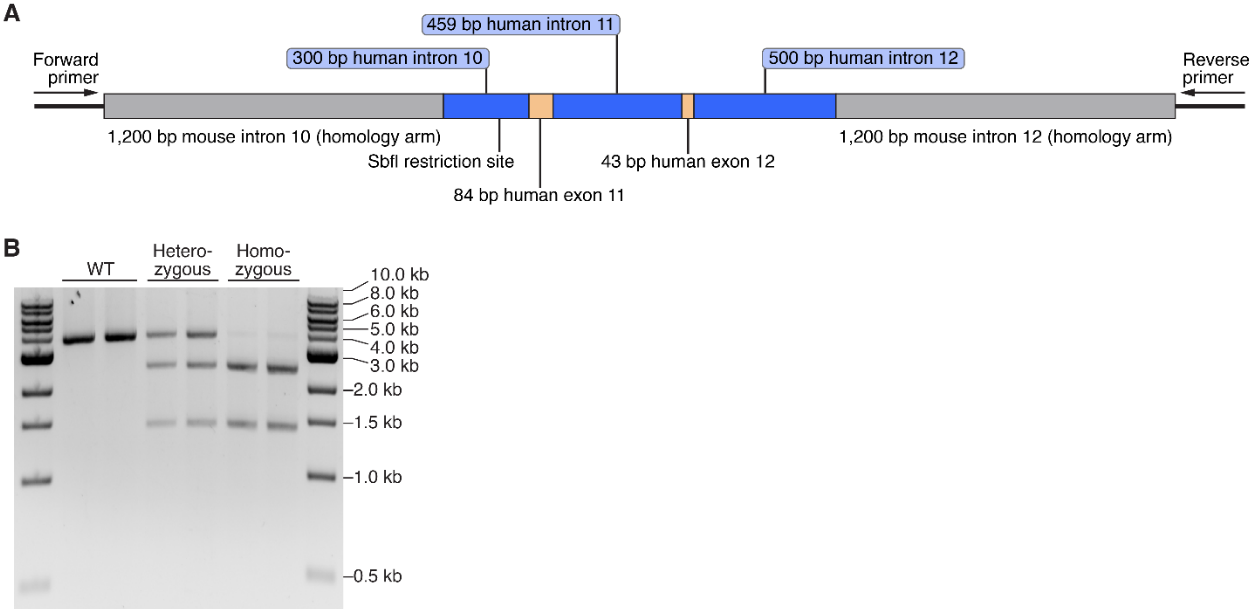
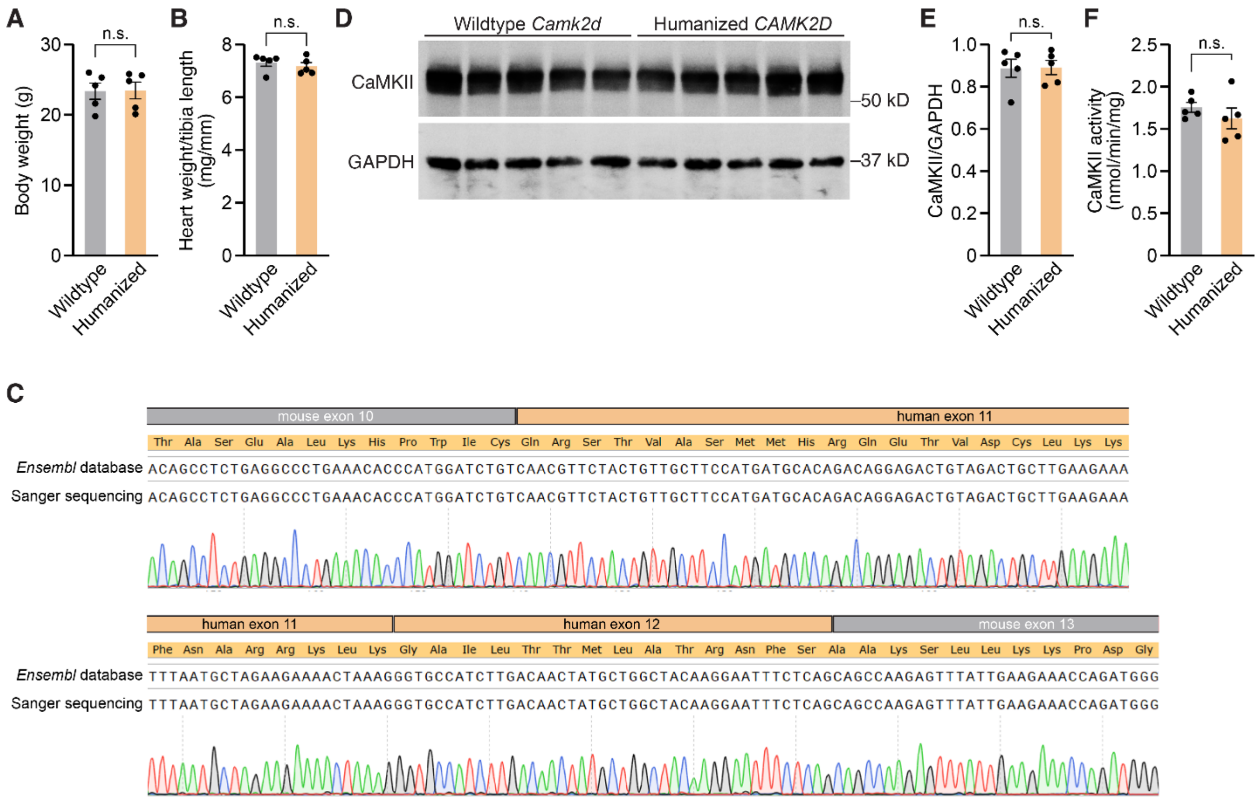
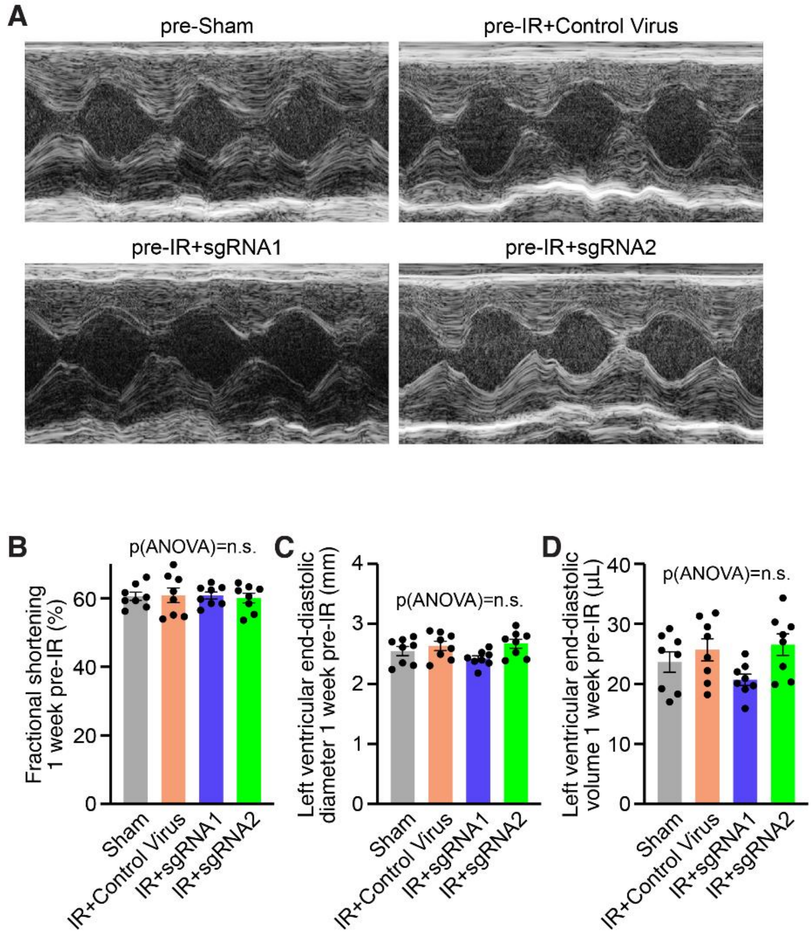
Supplemental Figures



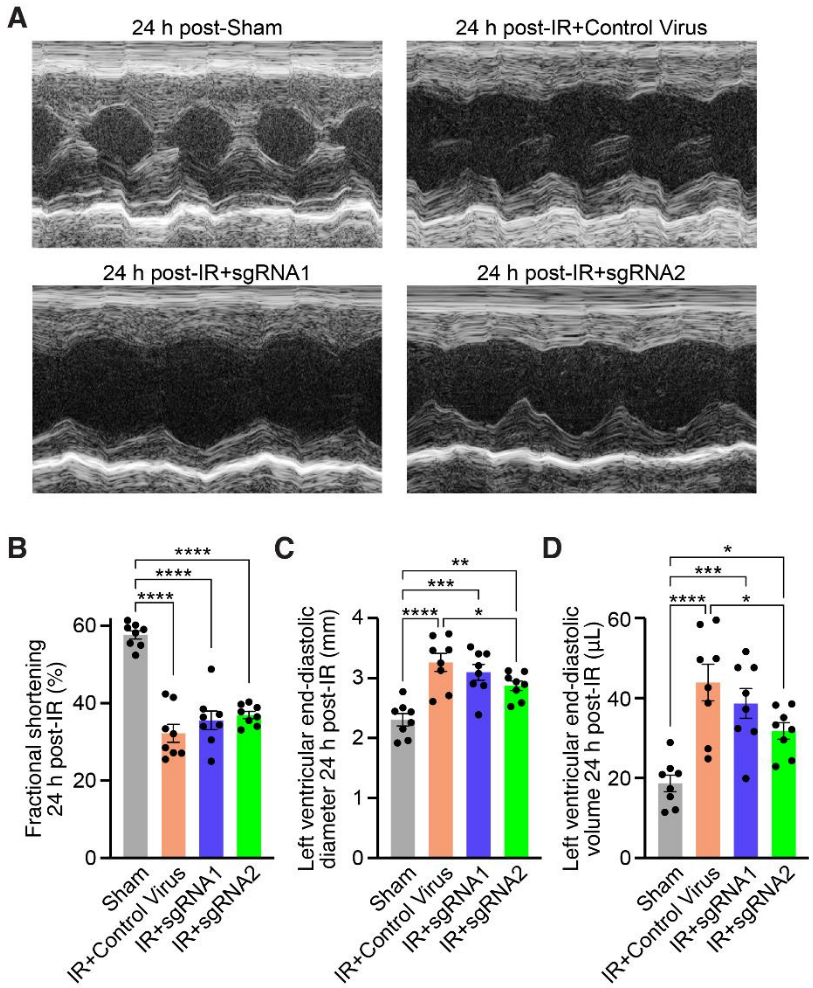
Supplemental Figure 1. Generation of a humanized *CAMK2D* knockin mouse model. A) Design of the donor template that was used to humanize the regulatory domain of CaMKIIδ. **B)** Representative genotyping gel of the SbfI-digested PCR product showing the pattern of wildtype (WT), heterozygous, and homozygous mice (n=2 each). Only the human sequence harbors a restriction site for SbfI.



Supplemental Figure 2. Basal characterization of homozygous humanized *CAMK2D* knockin mice. A) Mean body weight for female and male wildtype and humanized mice at 12 weeks of age (n=5 per group). **B)** Mean heart weight normalized to tibia length (n=5 per group). **C)** Sequencing of cardiac cDNA of a humanized mouse and alignment with the reference sequence reveals correct splicing of the human exons 11 and 12 between mouse exons 10 and 13. **D)** Western blot analysis of total CaMKII and GAPDH in cardiac tissue of 12-weeks old mice (n=5 per group). **E)** Mean densitometric analysis of total CaMKII normalized to GAPDH (n=5 per group). **F)** Mean CaMKII activity (n=5 per group). All data are individual data points with mean ± SEM and all replicates are individual mice. Statistical comparisons are based on Student's *t* (A and E and F) or Mann-Whitney test (B); n.s. – not statistically significant.



Supplemental Figure 3. Analysis of basal cardiac function and geometry one week before ischemia/reperfusion injury (IR). A) Representative M-mode traces of hearts from mice one week before either Sham, IR+Control Virus, IR+sgRNA1 or IR+sgRNA2 (echocardiography; in total n=8 per group). **B)** Mean fractional shortening one week pre-IR (n=8 per group). **C)** Mean left ventricular end-diastolic diameter one week pre-IR (n=8 per group). **D)** Mean left ventricular end-diastolic volume one week pre-IR (n=8 per group). All data are individual data points with mean ± SEM and all replicates are individual mice. Statistical comparisons are based on one-way ANOVA (B-D); n.s. – not statistically significant.



Supplemental Figure 4. Analysis of cardiac function and geometry 24 h after ischemia/reperfusion injury (IR). A) Representative M-mode traces of hearts from mice 24 h after either Sham, IR+Control Virus, IR+sgRNA1 or IR+sgRNA2 (echocardiography; in total n=8 per group). **B)** Mean fractional shortening 24 h post-IR (n=8 per group). **C)** Mean left ventricular end-diastolic diameter 24 h post-IR (n=8 per group). **D)** Mean left ventricular end-diastolic volume 24 h post-IR (n=8 per group). All data are individual data points with mean ± SEM and all replicates are individual mice. Statistical comparisons are based on one-way ANOVA post-hoc corrected by Holm-Sidak (B-D); * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$, **** – $p<0.0001$.

Supplemental Tables

Supplemental Table 1. PCR-primers used in this study.

Target	Primer	Sequence
Humanized-*CAMK2D*-mouse_ genotyping	Forward	TCTCAGTCCAGGATCCAGCCACT
	Reverse	GAATGGTGCAAAGGAACACGAGGTA
Human-*CAMK2D* (for human iPSCs and humanized mice)	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGTCAGTGTTGCATCTTGGT
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAAGAGCCCCAAAAAGAAT
Humanized-*CAMK2D*-mouse_ cDNA	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGGCACACCTGGGTATCTT
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCATCTGGTTTCTTCAATA
sgRNA1-off-target 1	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGGAAGAACATCAAGCCAGA
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAATTTTGGGGGACATGAACA
sgRNA1-off-target 2	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCAAAAACCCAGCTTCAAAA
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGTCAGCTTCACCCAATCAG
sgRNA1-off-target 3	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCAAGAAGGTTGAGCCTTTG
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAAAACAGCCACGATCAAT
sgRNA1-off-target 4	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCATATGACCACCCATTTC
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAACTAGGAGGAGCCACACC
sgRNA1-off-target 5	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTCCTGTCTCTGCTCCTTG
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACCTCCAAACTCACCCAGT
sgRNA1-off-target 6	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCACGAGCTGTTCCAGTAT
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCGGTCTGCTAAGTAGTGG
sgRNA1-off-target 7	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAACATTCGAGATGGCCTGT
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCCACACACTAATGCTGGA
sgRNA1-off-target 8	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGGTGGATTGCAAATTCCT
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCCTGCTGCCCTATAGATTG
sgRNA2-off-target 1	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATTCAGAATAGGCGGCTTCA
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCGTTTCCAGCCTTCATGT

sgRNA2-off-target 2	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGATGGCAACACAGCAGAG
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGAAAAACCTCGCTCAACT
sgRNA2-off-target 3	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGAGGTCTGTGGGTAAAGCA
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTATGTCCCCGGCTTACTGA
sgRNA2-off-target 4	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACCATTGCCATTCACCTCAT
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCCTGCTTCCTGATTCATA
sgRNA2-off-target 5	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTTCAAAGTGGGGCAAGTG
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCAGCATTTGGAAACCATA
sgRNA2-off-target 6	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAGGGGAACCAAAGGAAAG
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCAGGTACTCTGGCAGTTG
sgRNA2-off-target 7	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCAGAGTTCATCTCCCAGA
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATGCGGGTATCATGAAATGG
sgRNA2-off-target 8	Forward	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAATCAGTGTCAAGAGGCATCAA
	Reverse	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAATGACGTGGATGTGCAGAA
AAV-titer	Forward	GGAACCCCTAGTGATGGAGTT
	Reverse	CGGCCTCAGTGAGCGA

Supplemental Table 2. Sequences of sgRNAs and donor template used to humanize the regulatory domain of CaMKIIδ.

	Sequence	PAM
sgRNA-mouse-intron-10	TATACTGCTAAGTAAGGGGA	AGG
sgRNA-mouse-intron-12	ATTCATTTAGACTAATTACA	AGG
Humanization-template	ACCATGTGCTATCTACCCTTCTACACAACATGATGGGGAAATGGTTTCG TGTAGGGTAGAATAAGAGATGATGACAACTCAAGATTATTTCTTTCTCTG GCCAGCTCATGGGGCACCCAGTTGCAAAATCATGCCTATCTTGTCAGC ATTACTTATAGTTAAGTGTTTTAGAAATTCACACATTAATATCTCAGACAT GTTCACATCGAAACCAGTGGATCAAGTTAAAAATAAACATGACCAATTTT ATCTTGTTAGTTATTTCTTTGTTGCAGCAGACAGTGGTATGAATTAATCT ATTTTTTATATAATTTAACTTACATAATGGCAAATTTTATGAAACAATGCA CTGCTTATAGAAATGAGGGATATATTTGGATTTTCTCTATTTTACTCTGT GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTACTGTG TGTGTGTTTTAACACAGGATCTCATGTATACCAGGTTTCCCTTGCTCTTG CTATATATCTGAAGGTTGTCTAAAAGTCGTGATCCTTCCGCCTCTGCCT CTCTAGTGTTGGGATTAGAGGCATGTAGGAACACGTCTAGCTTAGTGTC TCTATTTTTAAAAACTCTTTACTAATTTCTATCTTTGAAGTTTCCGTCACC AGAATGGGATACAGTGACACCTGAAGCCAAAGACCTCATCAACAAAATG CTGACCATCAACCCTGCCAAACGTATCACAGCCTCTGAGGCCCTGAAA CACCCATGGATCTGTGTAAGTCACTTTCGCATGCACCCCAGGAGCCAA ACTGAAGGATAAACCCCTAGTCACTTTTCATAAGTTTAAAATAATCTTAC AGAAAAAGATGAACCTTAGTATTTAAGAAATAAATTTATAGTCGTGAAAA CCACCATTGCCAGTATAGCAAACTAAACTGCCCAATTTTCTAAGTATTGT CTGTAGTAAGATGAATGAGGTGTTCCTATGCCAGTAACTGATAAGACTC AAGAACAGGGACTAAGATAATGACTTGAATACTTACTGCACCCCAGAAG GGAAAATGTTACAAAGTCTAATTCTTTATCATTCAACAGCCTTAGAAACA TGTGAGGCTTAGAAGCTAGAAGTAGTTTTAGGGGCTACACGATGGCTG CAGTAGTGTGACTGTCTTGTAGAAGCCCTAAGGAAAAACCAAGGATATT GTAAGAGAGTTTGctcaaactcctgacctcgtgatccacccgccttggccttccaaagtgctggaa ttacaggcgtgacccaccgcgcccggccCATTTCTATACTTTCTATACGTCAGTGTTG CATCTTGGTCACAAGTAGTCTTGGCAAAACTAAAATTTTATTTTTAGGTA ACTTTGGCAATTGAATACTTAGAAGTTGATCCTGCAGGTGCAGCACCAT TAATAAATATTTGATGAAGTCAGGGACATTATTATACTTAGTGTGTTTTAA ATATTCTCAATTAAGTTACTTTTTCTCCTCTTCTTCTAGCAACGTTCTACT GTTGCTTCCATGATGCACAGACAGGAGACTGTAGACTGCTTGAAGAAAT TTAATGCTAGAAGAAAACTAAAGGTAAGAAATTTTACATTTTTATGGGGA GGTAGATTTGACAGACTAAGAAGTGAAATACTGTGTGAGGAAAATAACT TCTAGCAAAGGCTTTCAATTATGTAGACCTCAATACTCTCATTATTCTTTT TGGGGCTCTTGGGAAATGGAATATACACCAGAATCAAAACTATGGTGAA AAGCTGGAATCATCCTGGCAACACATGCTGTGGATCCTTCCTTCAGTGG AAAACAGCTGTGCAATTTGATAATCAGAATCTGGCCCGCACACTAGGGC CGTCCCAGCATCATGAGAGACAAAGGCCTTTGACTCTCAGGaaaccaaaa aacaaaaacaaacaataaaaaaTCCCCTAACTGAAAGTAGGGCCAGGAAAAGA TTTGTTATCAAAGACTCTATATTTGTTAGGTCCAGAATTTGCATAGGCAA CACTGACTTTTGTGTTCTAATTTTTTTCACAGGGTGCCATCTTGACAACT ATGCTGGCTACAAGGAATTTCTCAGGTACATGCATTGGGAACTCTGCTT CTTATCCCTTGGTCTTCTTTTTAAGTAGTCTCTGTTTTTTTGTCTCTGGAA	n/a

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