Supplementary Appendix

Supplement to: Lek A, Wong B, Keeler A, et al. Death after high-dose rAAV9 Gene therapy in a patient with Duchenne's muscular dystrophy. N Engl J Med 2023;389:1203-10. DOI: 10.1056/NEJMoa2307798

This appendix has been provided by the authors to give readers additional information about the work.

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SUPPLEMENTARY METHODS

Western blot on human muscle biopsies

Snap-frozen normal and DMD patient quadricep muscle biopsies were ground using a mortar and pestle over dry ice and lysed in RIPA buffer (50 mm HEPES pH 7.5, 150 mm NaCl, 5 mm EDTA, 1 mm EGTA, 15 mm p-nitrophenyl phosphate disodium hexahydral, 1% NP-40, 0.1% SDS, 1% deoxycholate and 0.025% sodium azide) with protease and phosphatase inhibitor cocktails added (Sigma). Lysates were incubated on ice for 1 h, vortexing halfway through, centrifuged at 16000g for 30 min at 4°C and the supernatants retained. Protein concentration was measured using a BCA protein assay kit (Bio-Rad). Lysates were separated by SDS–PAGE on 4-20% Tris–glycine gels (Bio-Rad) and transferred to PVDF membranes (Life Technologies). Membrane was blocked in 5% milk and 2% BSA in TTBS and then probed with rabbit polyclonal Dystrophin 6-10 antibody (Lidov, *et al*., 1990) at 1:750 followed by HRP-linked rabbit secondary (Cell Signaling, 7074). Blot was developed with Pierce ECL Plus (Thermo Scientific, 32132) on Blue Devil autoradiography film (Genesse Scientific, 30-101). Western blot was analyzed by ImageJ software.

Transcript expression analysis on post-mortem tissues

Primers:

hHPRT1-F: CATTATGCTGAGGATTTGGAAAGG
hHPRT1-R: CTTGAGCACACAGAGGGCTACA
129 bp

SaCas9_F: CTACGAGGCCAGAGTGAAGG
SaCas9_R: TCGGCCACGTATTTCTCTTC
192 bp

C7-F: GATCATGCGAAAGGGGAGC
C7-R: TCTCGCCAACAAGTTGACG
98 bp

Standard curves:

Standard Value (dSaCas9) = 4006.5765672931*e^(-0.6374394874*Ct)
Standard Value (hHPRT1) = 6720571.7317989400*e^(-0.6080430546*Ct)

C7 standard curve was not generated due to low expression.

Definition of expression levels of standard curves:

The dSaCas9 expression in 200 ng RNA from the heart tissue of mouse 3011 (clinical construct injected hDMD/D2-mdx mouse) and the hHPRT1 expression in 200 ng TaKaRa Human Heart RNA was defined as 1 for their standard curves, respectively.

RNA isolation

Tissues were added to 1 mL of TRIzol reagent (Life Technologies, 15596018) and homogenized until completely dissolved using a tissue homogenizer (Omni International, 10046-846). Once homogenized they were incubated at room temperature (RT) for 5 minutes (mins) to permit further dissociation. 200 µL of 1-Bromo-3-chloropropane (VWR, 10841- 634) was added and samples were vortexed vigorously then incubated at RT for 2-3 mins. Samples were centrifuged at 12,000 x g 4 °C for 15 mins to separate the RNA, DNA and protein phases. The aqueous RNA phase was carefully removed into a new 1.5 mL microcentrifuge tube and 500 µL of isopropanol (Sigma Aldrich, I9516) was added to precipitate the RNA. Samples were inverted to mix and incubated at RT for 10 mins before subsequent centrifugation at 12,000 × g for 15 mins at 4°C. The supernatant was removed from the pellet and the pellet was washed twice in 75% ethanol and centrifuged at 10,000 × g for 10 mins at 4°C. The supernatant was removed and the pellet was air dried for 10 mins at room temperature. The RNA pellet was resuspended in nuclease free water depending on pellet size (50–800 µL). Concentration and purity were measured using NanoDrop (Thermofisher Scientific, ND2000).

DNase Treatment

Residual genomic DNA in the RNA samples were removed using the Invitrogen ezDNase kit (Thermofisher Scientific, 11766051). Briefly, 2 µg RNA samples were diluted to 8 µL with Nuclease free water. 1 µL ezDNase and 1 µL reaction buffer were used for each reaction. The samples were incubated at 37°C for 10 mins and then the DNase was deactivated at 70°C for 3 mins in a ProFlex thermal cycler (Applied Biosystems, 4484073).

One-step RT-qPCR

The RT-qPCR reaction used 20 ng RNA (dSaCas9) or 200 ng RNA (hHPRT1; C7) as a template and the reaction contained iTaq Universal SYBR Green Reaction mix and Reverse transcriptase (BioRad, 1725151), forward and reverse primers (final concentration of 250nM each) and nuclease free water to a total volume of 20 µl. The qPCR reaction was prepared in Multiplate 96 well PCR plates (low profile) (BioRad, MLL9601) which were sealed using Microseal 'B' PCR Plate Sealing Film, adhesive (BioRad, MSB1001).

Reagent	**Amount**
20 ng RNA or 200 ng RNA	x µL
10 µM Forward & Reverse primer mix	1 µL
Reverse transcriptase	0.25 µL
Reaction mix	10 µL
nuclease free water	Up to 20 µL

The qPCR was performed on a CFX96 C1000 touch real time PCR detection system (BioRad, 1855196) using a 2-step amplification plus melt curve with the following parameters. qPCR triplicates were performed for each reaction.

Temperature	Time	Number of Cycles
50 °C	10 mins	x1
95 °C	3 mins	x1
95 °C	10 secs	x39 plus plate read
57 °C	1 min	

To check for primer specificity an additional melt curve analysis step was performed starting at 65°C for 0.05 seconds with 5-degree increments until 95 °C with a plate read step to capture data.

For data analysis Ct values were collected. The expression was quantitated using the standard curves (dSaCas9) or ddCT (C7).

Protein Extraction and Quantitation

Mouse tissues were harvested 4 weeks post-injection. Both human and mouse tissues were homogenized in 250–500 µL of ice-cold protein extraction lysis buffer made by adding 10 µL of protease inhibitor cocktail (Sigma Aldrich, P8340-1ML) to 1 mL of RIPA lysis and extraction buffer (Pierce, PI89900). After 20 minutes of incubation on ice, samples were vortexed again, and sonicated by Bioruptor® Plus Sonication System (Diagenode, B01020001) for 13 rounds of 30s on and 30s off. Samples were centrifuged for 30 minutes at 16,000 × g at 4 °C in a refrigerated centrifuge (Eppendorf, 5404). The supernatant was transferred to a clean 1.5 mL microcentrifuge tube and placed on ice until progressed to the quantitation.

Protein was quantitated using the BioRad DC Assay II kit (BioRad, 5000112). Using the BSA reagent from the kit, seven standards are prepared from 0–1.5 mg/mL in the protein extraction buffer from above for creating a standard curve. Measurements were done according to the instruction manual, and absorbances at 750nm were read on a plate reader (BioRad, #1681135). Concentration is calculated relative to the standard curve.

SDS-PAGE and western blotting for mouse and post-mortem tissue

Appropriate amount of protein extraction lysis buffer from above was added into a specific loading amount of each protein sample to achieve an equal volume for each sample, which was then prepared in 4x Laemlli sample buffer (BioRad, 1610747). Samples were mixed by a brief vortex and then heated at 95°C on a heat block (BioRad, 1660571) for 5 minutes. Samples were cooled down on ice and then centrifuged briefly at max speed to ensure equal loading (Eppendorf, 5404). 3.5 µL of PageRuler Plus prestained protein ladder (ThermoFisher Scientific, 26619) and all of the samples were then loaded onto a 4-15% mini-protean TGX stain free protein gel (BioRad, 4568084) and placed into the Mini-PROTEAN tetra cell electrophoresis system (BioRad, 1658000). SDS-PAGE was performed at 100V for 80 minutes using a PowerPac HC power supply (BioRad, 1645052). Upon completion of the SDS-PAGE, the gel was removed and transferred onto nitrocellulose membrane (BioRad, 1704158) by the TransBlot Turbo System (BioRad, 1704150EDU) using the 7-minute transfer program (2.5 A constant, up to 25 V variable).

Membrane blocking buffer was made of 5% Omniblock non-fat dry milk (American Bio, AB10109-00100) in 1× TBS-Tween 20 (TBS-T). The membrane was then blocked for 1 hour in membrane blocking buffer. Primary antibody Anti-CRISPR-Cas9 (Abcam, ab203943) was made up 1:1,000 in membrane blocking buffer and the membrane was incubated in the primary antibody overnight

at 4°C whilst rocking to equally distribute antibody (BenchRocker 2D Genesee Scientific, 31-201). The following day the membrane was washed 5 times for 5 minutes in TBS-T and incubated in secondary anti-rabbit HRP conjugated secondary antibody (Cell Signaling Technology, 7074S) at 1:2000 in membrane blocking buffer for 2 hours at room temperature. Membrane was washed again 5 times for 5 mins at room temperature in TBS-T prior to developing with Clarity Max ECL substrate (BioRad, 1705062). Using the Chemidoc Imaging system (BioRad, 12005606), both chemiluminescent and colorimetric images were taken to observe protein expression and size of bands. To determine equal protein loading, the membrane was stripped for 15 minutes in Restore PLUS western blot stripping buffer (ThermoFisher Scientific, 46430) at room temperature, washed in 1x TBS-T and then re-blocked in membrane blocking buffer for 1 hour. Vinculin was used as a loading control, thus the membrane was incubated in primary antibody (Sigma Aldrich, V9131-100 µl) at 1:80,000 dilution overnight at 4 °C whilst rocking to equally distribute antibody. Washes and secondary were performed as described using Anti-mouse HRP conjugated secondary antibody (Cell Signaling Technology, 7076S). Membrane was developed using the Clarity ECL substrate (BioRad, 1705060) and imaged using the Chemidoc Imaging system (BioRad, 12005606).

CBA assay

For the detection of cytokines in serum, Cytometric Bead Array (CBA) assay was performed using Biolegend LEGENDplex HU Essential Immune Response Panel (13-plex) with V-bottom Plate (Biolegend, Cat# 740930) or Biolegend LEGENDplex Human Type 1/2/3 Interferon Panel (5-plex) with V-bottom Plate (Biolegend, Cat# 740396) following the manufacturer's recommendations. For the detection of cytokines in peri-cardiac fluid, Cytometric Bead Array (CBA) assay was performed using Biolegend LEGENDplex Human Type 1/2/3 Interferon Panel (5-plex) with V-bottom Plate (Biolegend, Cat# 740396) following the manufacturer's recommendations. Serum samples from the patient were diluted 1:2. The peri-cardiac fluid sample from the patient was not diluted for the assay. Data were acquired on a BD LSRII and analyzed using the LEGENDplex Data Analysis Software Suite.

ddPCR

Digital droplet PCR (ddPCR) was performed using 5ng of gDNA and a human RPP30 reference primer/probe as well as the following primers and FAM labeled probe for the CK8e promoter:

Forward primer: accctgcatgccatgttc

Reverse primer: ctgacttgctcactggttcc

Probe: cccgccagctagactcagca

Samples were run on the Bio Rad QX200 system according to the manufacturers recommendations. To be considered valid, each well is required to have at least 10,000 accepted droplets, of which 100 must be negative.

qPCR for vector biodistribution

To measure vector biodistribution, harvested tissue was processed for genomic DNA, using the Gentra Puregene Kit (Qiagen) as per manufacturer's recommendations. Vector genome copy numbers were measured by qPCR in 20 ng of total DNA, using the following primers and probes for the BGH polyA in the vector:

forward primer CCTCGACTGTGCCTTCTAG;

reverse primer TGCGATGCAATTTCCTCAT;

probe 56-FAM/TGCCAGCCA/ZEN/TCTGTTGTTTGCC/3IABkFQ.

All samples were compared to a plasmid-based standard curve and a viral vector internal control for quality assurance. The primer-probe mix was acquired from IDT, and the reaction was run using TaqMan Gene Expression Master Mix (Applied Biosystems) in a CFX Opus 96 qPCR system (Bio-Rad).

ELISPOT

An ELISpot assay detecting the secreted INFg was performed on patient PBMCs to measure the cellular immune response to the AAV9 capsid and dSaCas9 transgene. Peptides spanning the transgene and the VP1 of the capsid, consisting of 15-mers that overlap by 10aa (mimotopes), were divided separately into pools (five pools for the transgene and three pools for the capsid). The cells are stimulated for 48hrs with the peptides, a negative control (media only), and a positive control (CD3/CD28, Mabtech) for cytokine secretion. Spots numbers were analyzed and counted using the Mabtech IRIS reader. To consider the cellular immune response positive, the number

of spot-forming units per 10^6 cells must be greater than 50 and 3-fold higher than the CD3/CD28 positive control.

Clinical trial

The patient provided written informed consent for the clinical trial study which was sponsored by Cure Rare Disease and performed in accordance with protocols approved by the institutional review board at University of Massachusetts Chan Medical School. Clinical-grade plasmid and vector were manufactured at Aldevron and Andelyn, respectively. The trial was conducted at University of Massachusetts Chan Medical School; imaging and safety labs were performed at U Mass Memorial Hospital Medical center; AAV9 antibody testing was done at Athena Diagnostics.

Autopsy

A limited autopsy was consented by the patient's family in which liver, brain, skeletal muscle, lungs, heart tissue were evaluated by the pathology department of Boston Children's Hospital. Postmortem examination was performed 19 hours following death. Macroscopic and microscopic examination was conducted for each organ. Routine H&E stains were evaluated for representative sections of each organ. Special stain (Masson trichrome for fibrosis) and immunohistochemical stains (CD3, CD20 and C4d for T cells, B cells, and complement deposition, respectively) were performed in selected sections of cardiac tissue. Periodic acid-Schiff (PAS) stain was performed on selected lung sections to confirm hyaline membrane deposition. In addition, DNA, RNA and protein were extracted from tissues for vector genome copy number, transgene transcript and protein quantification, respectively

SUPPLEMENTARY FIGURES

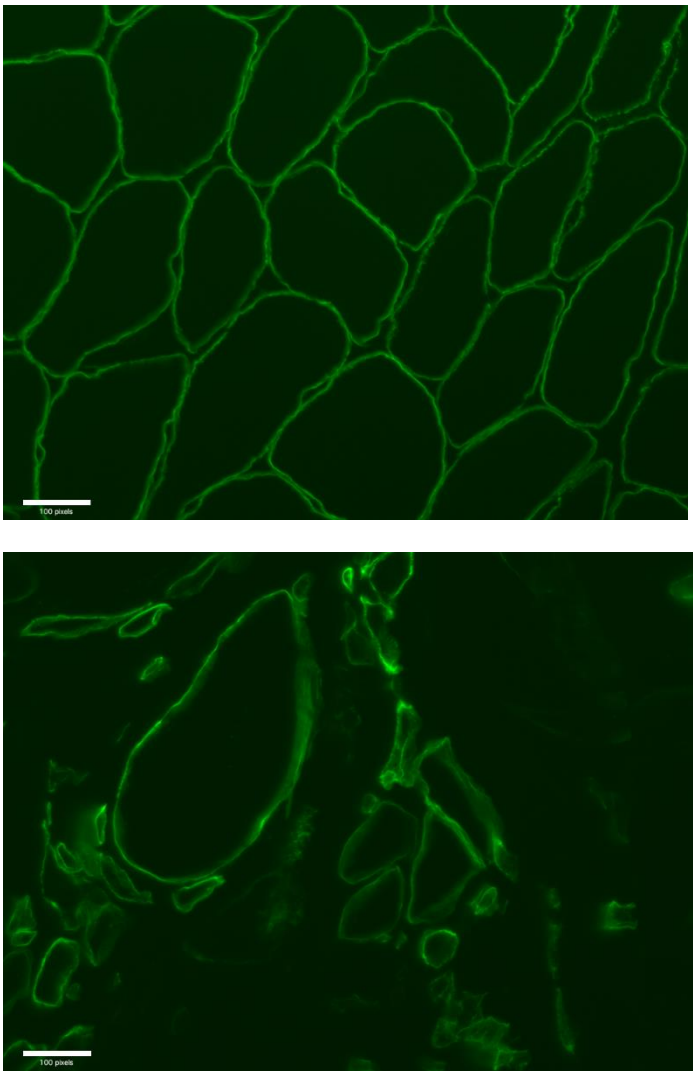
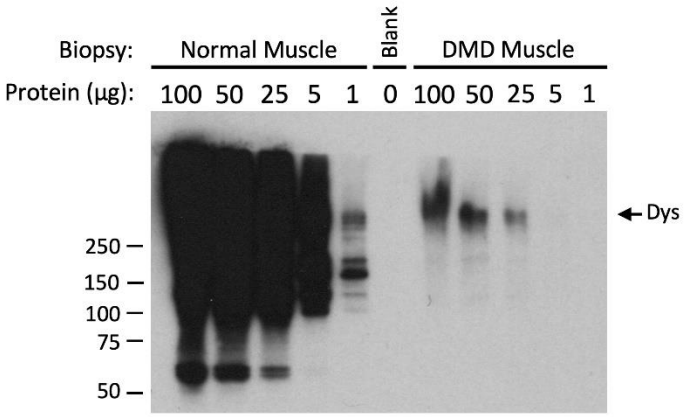


Figure S1: Dystrophin immunostaining in normal and DMD patient muscle. Frozen muscle tissue section from a de-identified unaffected individual (top) and the proband (bottom) immunostained for dystrophin. Dystrophin staining is visible at the myofiber sarcolemma in the unaffected control sample and also in several myofibers of different sizes in the proband. The positive staining was detected using antibodies to the dystrophin rod domain and C-terminus (CAP 6-10 antibody generated in Kunkel Lab).



Lane	Normal (PD)	DMD (PD)	Normal Adjusted (PD)	(DMD/Normal Adjusted)*100	Average %	Stdev	SE
1µg Normal	7971.823						
100µg DMD		20485.409	797182.300	2.570			
50µg DMD		12675.581	398591.150	3.180	**2.879**	0.305	0.176
25µg DMD		5752.225	199295.575	2.886			

Figure S2: Dystrophin expression via western blot in normal and DMD patient muscle. Top: Western blot of normal and DMD patient quadricep muscle biopsies shows significantly diminished dystrophin expression in DMD patient compared to normal muscle. Bottom: Western blot quantification indicates ~2.9% dystrophin protein expression in DMD patient compared to normal muscle. PD = pixel density as quantified in ImageJ.

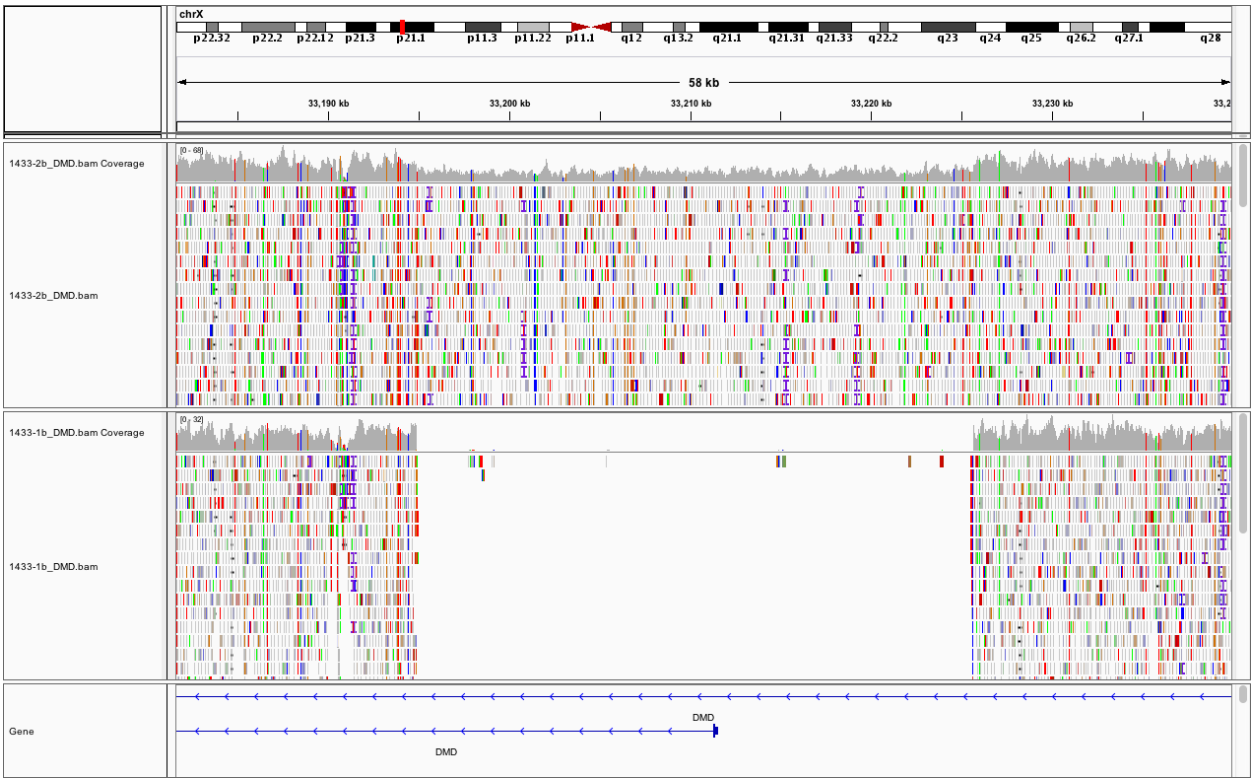


Figure S3: Whole genome sequencing of patient and mother DNA. Whole genome sequencing reveals a 30 kb hemizygous deletion (GRCh38 chrX: 33,194,894-33,225,637) encompassing the promoter and exon 1 of the muscle (*Dp427m*) isoform (bottom panel). The mother of the patient (top panel) is heterozygous for the deletion revealed by 0.5× coverage in the histogram in the overlapping region and split read support.

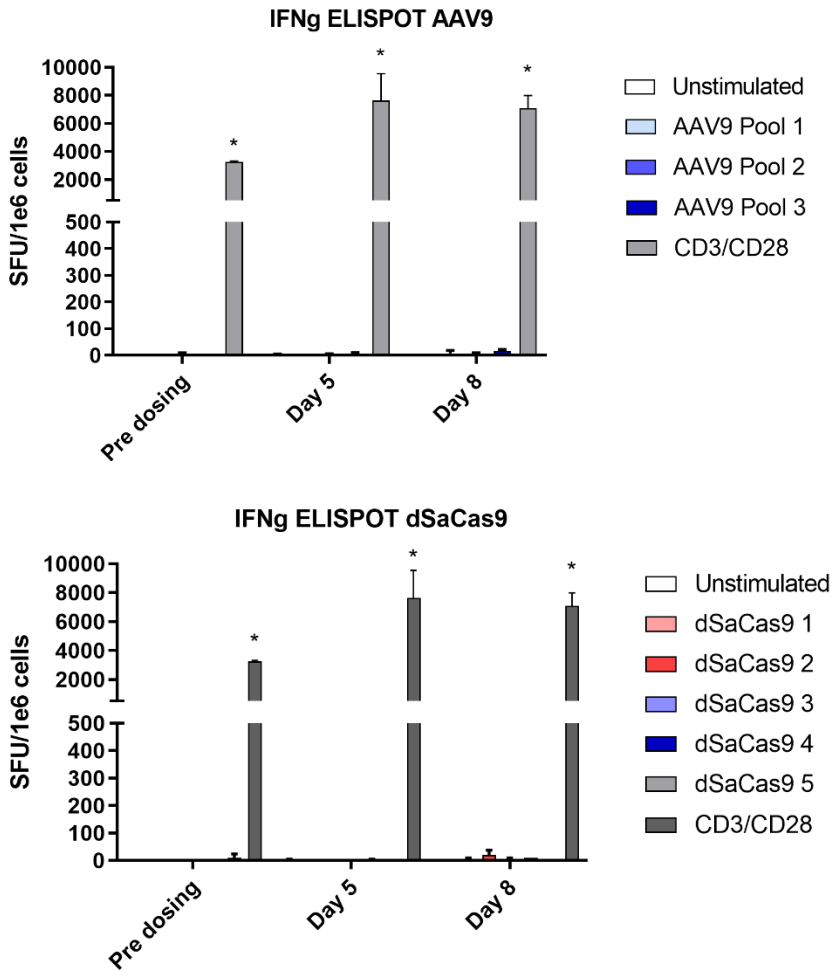


Figure S4: Interferon-gamma ELISPOT assays for AAV9 and SaCas9 in patient samples. PBMCs isolated from the patient at different time-points in life were stimulated by either AAV9 (left) or dSaCas9 (right) peptide pools to assess T-cell responses by interferon-γ ELISPOT assay. Negative controls were unstimulated cells, and CD3/CD28 stimulation was used as positive control. Data was run in technical triplicates and reported as mean ±SD. Significance designated by * represents 3x negative control. Peripheral blood mononuclear cells (PBMCs), spot forming unit (SFU) standard deviation (SD).

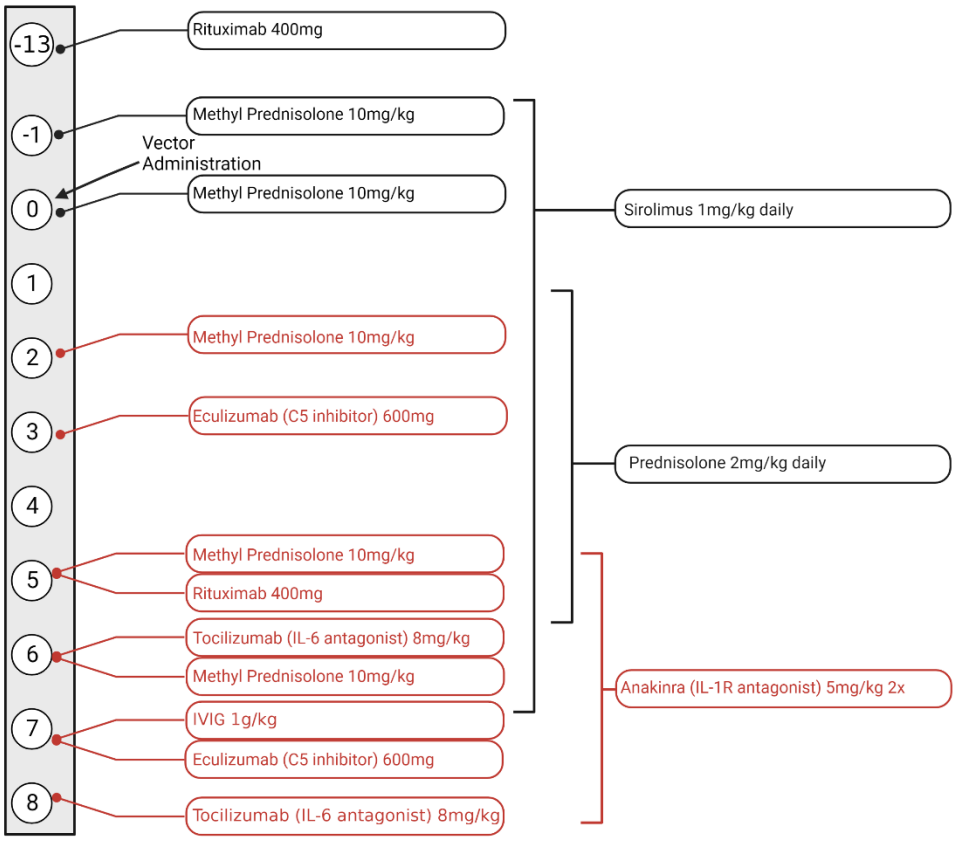


Figure S5: Timeline of prophylactic immune suppression administered during clinical trial.

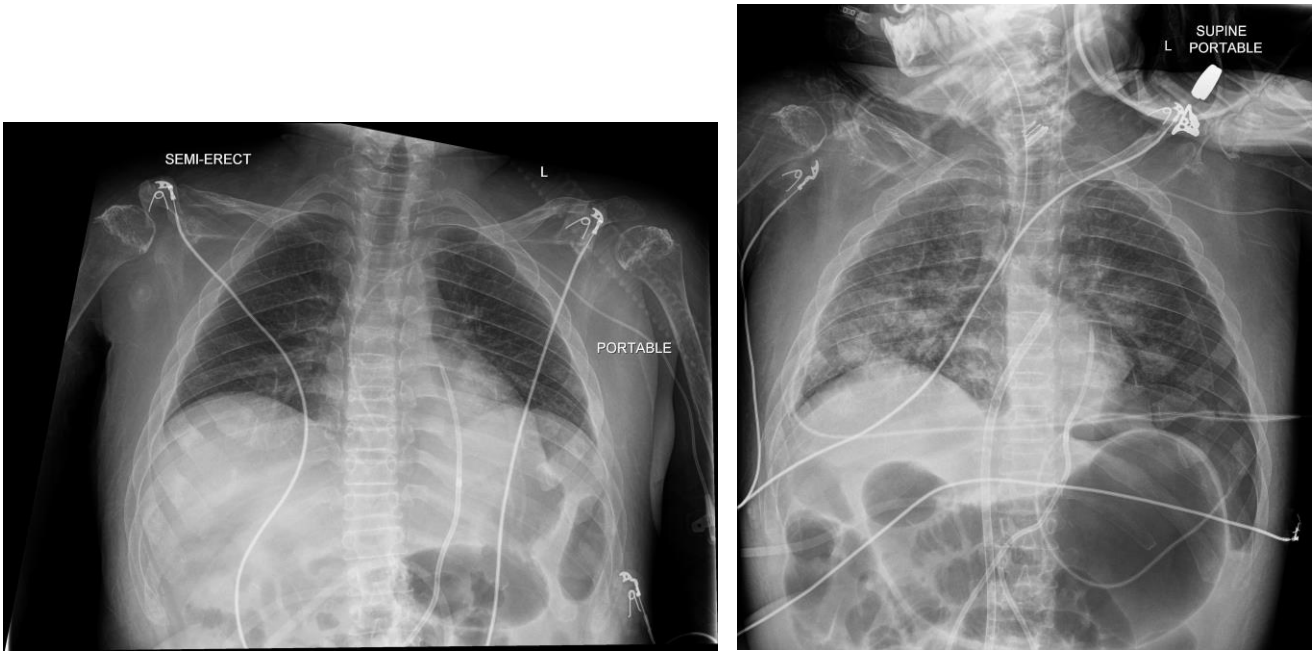


Figure S6: Chest X-ray at two timepoints on day 6 post-dosing. Rapid evolution of Chest X-ray findings of ARDS developing in an 8.5 hr interval on day 6 (left: T0, right: T8.5 hrs).

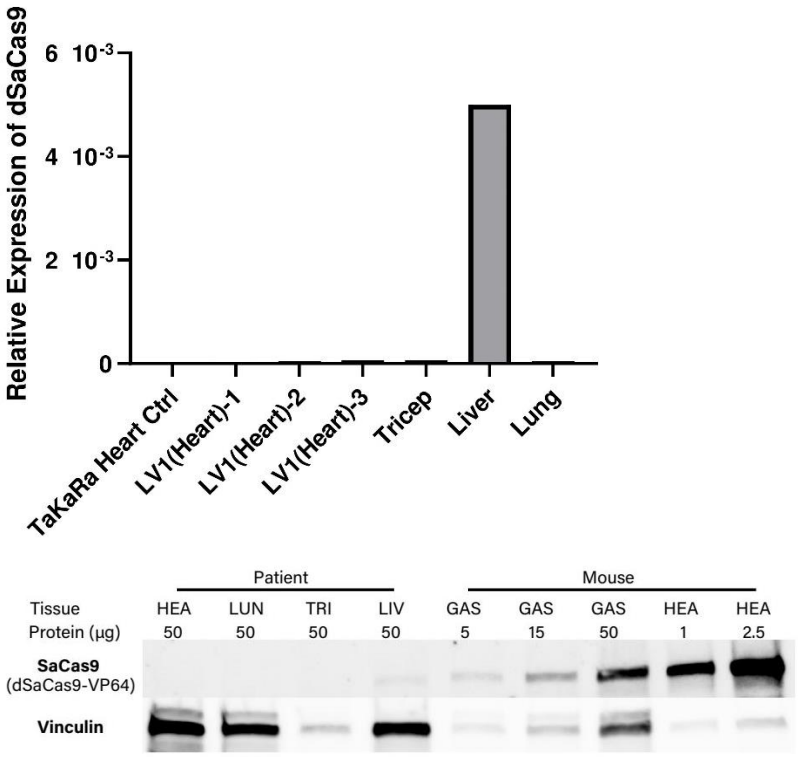


Figure S7: Transcript and protein expression of dSaCas9 in patient tissues.

Top: RNA expression levels of d*Sa*Cas9 were measured by RT-qPCR. Heart tissue was sampled in three different locations. Bottom: SaCas9 expression in post-mortem tissue compared to AAV-injected mice at similar dosage. Protein expression in GAS and HEA mouse tissue is from 8-week time point compared to 8-day post-treatment from patient tissues. Vinculin was used as a loading control but showed variable relative expression in non-muscle tissues. HEA, heart; LUN, lung; TRI, triceps; LIV, liver; GAS, gastrocnemius.

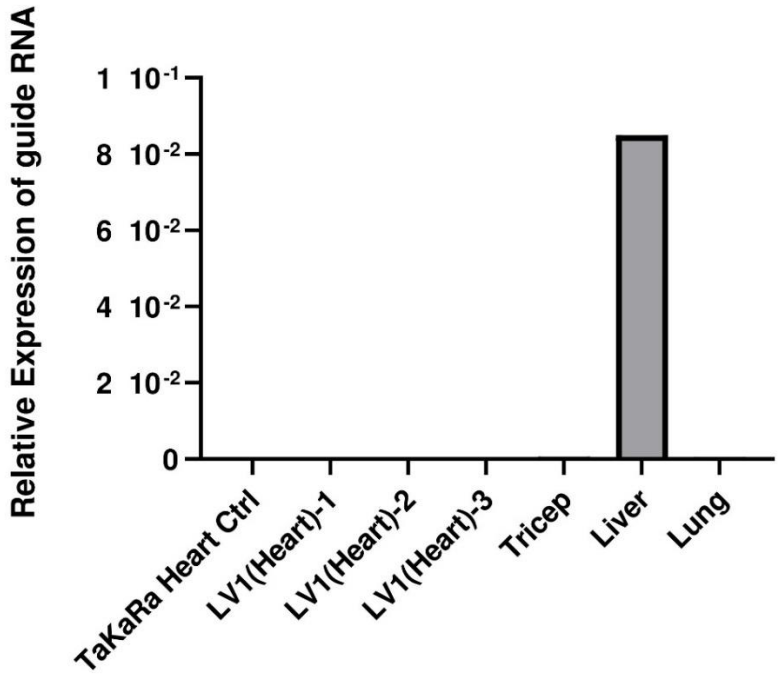


Figure S8: Expression of guide RNA in post-mortem tissues. Expression level of the guide (C7) was measured by RT-qPCR and normalized to the housekeeping gene hHPRT1. qPCR triplicates were performed for each reaction. Values reported were quantified with ddCT. Left ventrical (LV) was sampled in 3 different places.

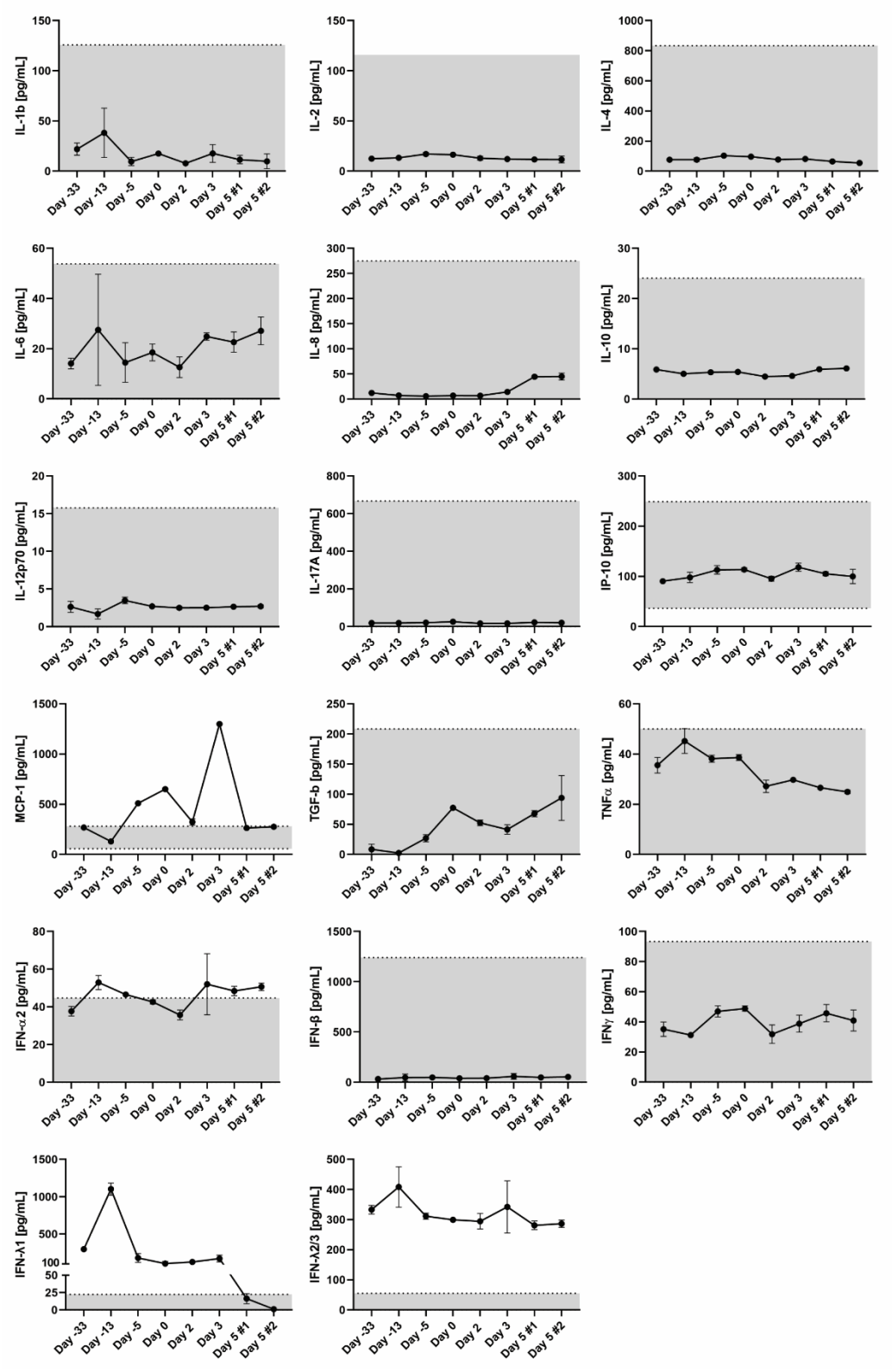
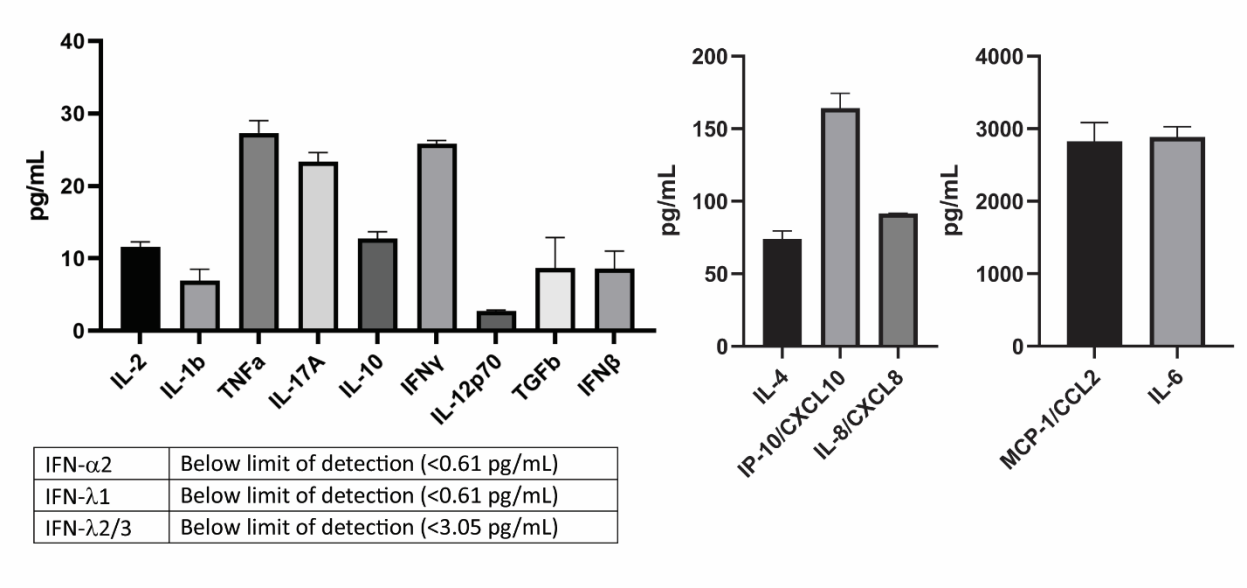


Figure S9: Serum Cytokines over time. Multiplex Cytokine bead assay of serum from patient before and after gene therapy. Error bars represent technical replicates.



IFN-α2	Below limit of detection (<0.61 pg/mL)
IFN-λ1	Below limit of detection (<0.61 pg/mL)
IFN-λ2/3	Below limit of detection (<3.05 pg/mL)

Figure S10: Pericardial Fluid Cytokines. Multiplex Cytokine bead assay of Pericardial fluid removed from patient day 6 post-treatment. Error bars represent technical replicates.

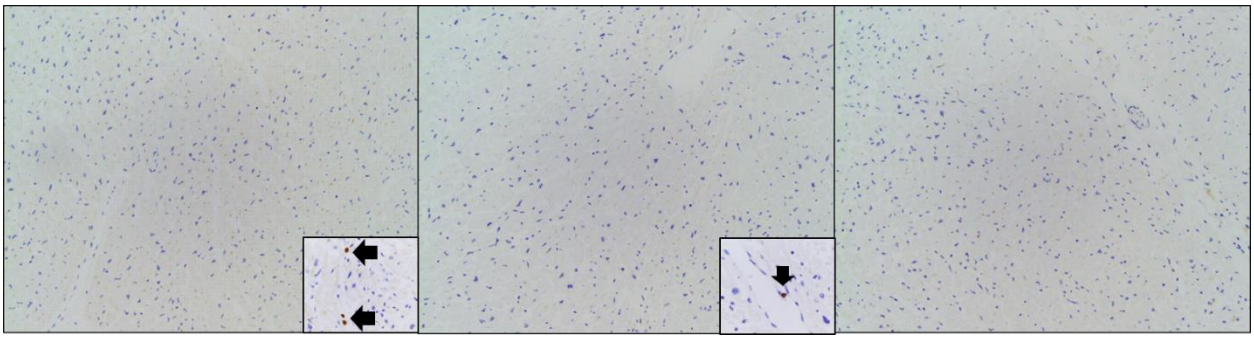


Figure S11: Post-mortem immunohistochemistry on cardiac tissue. There are no increased lymphocytes, either T or B cells, in the heart tissue by CD3 and CD20 immunohistochemistry, respectively (10×). Insets show internal positive control of rare intravascular T cells (CD3, 20×) and B cell (CD20, 20×), pointed out by arrows. C4d (10×) stain is also negative in the heart tissue. The findings argue against inflammatory and complement-mediated processes.

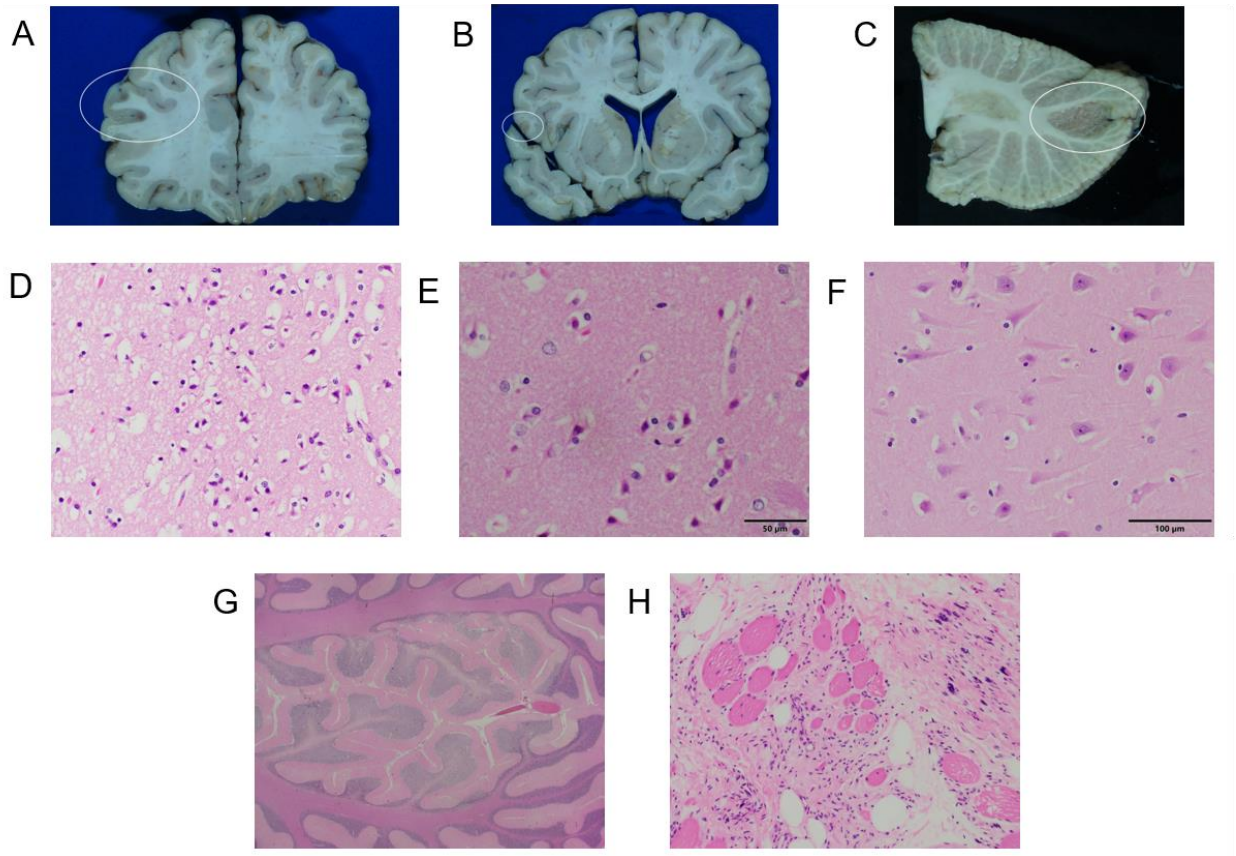


Figure S12: Supplemental Histology Images. A) Gross examination of a coronal section of the frontal lobe showing discolored foci (white ellipse) of infarction in the left inferior frontal gyrus. **B)** Gross examination of a coronal section of the frontal lobe showing discolored foci (white ellipse) of infarction in the left inferior frontal gyrus. **C)** Gross examination of the cerebellum showing discolored foci (white ellipse) of injury between superior and inferior cerebellar artery distributions, which is characteristic of watershed infarction. **D)** Histological analysis (40×) of the frontal cortex shows scattered neurons with dark pyknotic nuclei and markedly hypereosinophilic cytoplasm, indicating hypoxic ischemic injury days- to hours-old. Additionally, the neuropil is finely vacuolated suggesting cerebral edema. **E)** Histological analysis (60×) of the caudate nucleus shows scattered neurons with dark pyknotic nuclei and markedly hypereosinophilic cytoplasm, indicating hypoxic ischemic injury days- to hours-old. **F)** Histological analysis (40×) of hippocampal pyramidal cells shows many pale with indistinct nuclei and mild hypereosinophila, reflecting recent hypoxic ischemic injury. **G)** H&E examination (1.25×) of the focal injury seen in

the cerebellum (C). Note the diffuse pallor in the central area of the cortex affecting the internal granule cell layer and, less conspicuously, the molecular layer, in contrast to the surrounding periphery. **H)** H&E examination (20×) of a paraffin-embedded section of triceps muscle showing severe fibrosis, fatty replacement, and depletion of myofibers, with the few remaining fibers being abnormally round with internalized nuclei. A few severely atrophic fibers and “nuclear bags” are present. All features are consistent with end stage dystrophic myopathy.

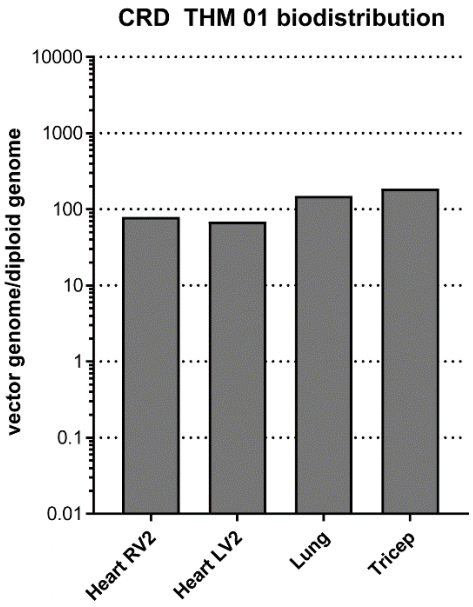


Figure S13: ddPCR of post-mortem tissues. ddPCR for vector genomes confirmed qPCR values for heart (RV = right ventricle, LV = left ventricle), lung and triceps muscle, but liver vector genome number was above upper limit of detection.

SUPPLEMENTARY TABLES

Table S1: Certificate of analysis for GMP-grade AAV manufactured by Andelyn

Product Name:	CRD-TMH-001
Product Description:	The biological product is a non-replicating, serotype AAV9 single-stranded (ss) recombinant adeno-associated virus (rAAV) designated CRD-TMH-001. The vector plasmid Construct1_singleVP64_C7 contains the transgene dSaCas9-VP64 which encodes staph aureus Cas9 protein/guide RNA sequences. Expression of the transgene cassette is under the control of the Ck8e promoter. The biologic product will be administered to enable in vivo expression of Dp427c.
Lot Number:	G0071A0122
Concentration:	4.07E + 13 vg/mL
Date of Manufacture:	031122 (Vialing Date)
Expiration Date:	Pending Stability Testing
Storage Conditions:	≤ -60 °C
Yield (for Clinical Use):	306 Vials at 1.75 mL per vial

Test Description	Acceptance Criteria	Result
Sterility - USP <71>	No Growth or Negative	Negative
Bacteriostasis/Fungistasis - USP <71>	Pass	Pass
Endotoxin	< 2.83 EU/mL	< 0.062 EU/mL
Physical Titer	> 2.5E+13 vg/mL	4.07E+13 vg/mL
Infectious Unit Titer	Report Result	1.7E+09 IU/mL
Total Protein	Report Result	352.711 µg/mL
Purity	Purity > 90% with no single impurity > 2%	Total Purity = 100% Impurity Bands: 82.9 kD < 1% 76.5 kD < 1% 67.4 kD < 1% 49.9 kD < 1% 46.7 kD < 1% 43.4 kD < 1% 41.7 kD < 1% 33.9 kD < 1% 32.3 kD < 1% 31.5 kD < 1% 29.6 kD < 1%
Identity (DNA)	DNA matches the expected sequence	DNA matches the expected sequence
Identity (Protein)	Positive for AAV Capsid Protein	Positive for AAV Capsid Protein
Osmolality	388 – 426 mOsm/kg (mOsm)	408.7 mOsm/kg
pH	8.0 ± 0.5	pH = 7.96

Table S2: Anti-AAV9 Neutralizing Antibody levels (Athena Diagnostics)

	D-30	D-14	D-7	D1	D2	D3
Anti-AAV9	<1:25	<1:25	<1:25	<1:25	<1:25	<1:25

Table S3: Patient lab results

Chemistries

CRD-TMH-001		D-30	D-14	D-1	D2		D3	D4	D5		D6	D7			D8					D9	
		8/31/2022	9/20/2022	10/3/2022	10/5/2022		10/6/2022	10/7/2022	10/8/2022		10/9/2022	10/10/2022			10/11/2022					10/12/2022	
	Umass Normal Ranges	10:08am	10:26	11:48am	8:34am	8:42am	9:06am	6:22am	5:11am	12:43 pm	2:31am	5:42am	4:55pm	7:07pm	12:30am	4:40am	9:03am	4:09pm	11:29pm	03:04am	BCH Normal Ranges
Comprehensive Metabolic Panel																					
Sodium	135-145 mmol/L	134		136		136	139	137	137		137	132	142	145	145	148		148		142	135-138 mmol/L
Potassium	3.5-5.3 mmol/L	4.1		3.4		3.5	4	3.8	4.2		3.8	4.5	5.8	4.7	3.96	2.84		3.54		3.87	3.2-4.5 mmol/L
Cloride	97-100 mmol/L	98		100		101	103	103	102		99	97	102	107	101	101		102		102	96-109 mmol/L
CO2	24-32 mmol/L	26		26		25	21	26	27		30	22	24	11	9	17		30		28	22-30 mmol/L
Anion Gap	5 to 15	10		10		10	15	8	8		8	13	16	27	35	30		16		12	7-14 mmol/L
Glucose	70-99 mg/dL	91		128		98	71	129	85		99	108	233	69	168	228		178		136	70-99 mg/dL
Creatinine	0.60-1.30 mg/dL	<0.20		<0.20		<0.20	<0.20	<0.20	<0.20		<0.20	<0.20	0.32	<0.20	0.22	0.21		0.28		0.33	0.6-1.3 mg/dL
Calcium	8.7-10.7 mg/dL	9.1		9.5		8.3	8.4	7.8	7.6		7.7	7.6	14.2	6.7	8	6.9		8.2		7.7	8.4-10.5 mg/dL
Total Protein	6.0-8.0 g/dL	6.3		5.8		5.3	5	5	4.8		4.7	4.9	3.2		4.1		4.2	4.5		5.7	5.5-8.2 g/dL
Albumin	3.5-4.8	4.2		4		3.6	3.6	3.3	3.2		3.2	2.9	2		3.2		3.2	3.2		2.7	3.0-4.6 g/dL
Bilirubin, Total	0.3-1.2 mg/dL	0.4		0.4		0.4	0.3	0.2	0.2		0.1	0.3	0.3		0.4		0.4	0.3		0.5	0.3-1.2 mg/dL
Bilirubin, Direct	<=0.4 mg/dL			<0.1		0.1	0.1													<0.2	0.0-0.4mg/dL
Alk Phos	30-115 U/L	35		24		19	19	18	17		22	20	41		45		35	37		32	30-120 U/L
AST	10-40 U/L	36		31		53	62	57	50		80	140	143		485		300	330		308	2-40 U/L
ALT	10-40 U/L	40		37		43	48	48	41		49	63	58		150		96	85		52	3-30 U/L
Bun	7-23 mg/dL	9		6		11	18	9	15		13	16	16	14	18	19		18		22	7-18 mg/dL
eGFR	>=60 NA	133		133	122			131			130		>90								
Cystatin C	0.52=1.35 mg/L	0.64		0.64	0.77			0.66			0.67										
Magnesium	1.6-2.4 mg/dL	2.2		2		2.1	2.1	2.1	2.1		2.1	2			2.4	2	2.1	2.1		1.8	1.6-2.6 mg/dL
Phosphorus	2.5-4.5 mg/dL	4.1		4.9		3.5	3.4	2.7	2.9		3	3.4			5.4	5.6		7		6.2	2.7-4.9 mg/dL
GGT	7-50 U/L	20		15		14			13	16	15								15		12-55 U/L
CRP	<10.0 mg/L				1.1							1.9						2.68		6.65	<=0.50
Sed Rate	<15 mm/hr						3					5							3		
hsCRP	<1.0		0.4																		
Calcium Ionized	4.6-5.3 mg/dL							4.5			4.5										
Procalcitonin																				8.94	<=0.09
IgA	47 - 310 mg/dL	47																			
IgG	600 - 1640 mg/dL	381																			
LDH	110 - 240 U/L	262																1029			100-210U/L
Ferritin	23-336 ng/mL											5777								4281	10-320ng/mL

Chemistries

CRD-TMH-001		D-30	D-14	D-1	D2		D3	D4	D5		D6	D7			D8					D9	
		8/31/2022	9/20/2022	10/3/2022	10/5/2022		10/6/2022	10/7/2022	10/8/2022		10/9/2022	10/10/2022			10/11/2022					10/12/2022	
	Umass Normal Ranges	10:08am	10:26	11:48am	8:34am	8:42am	9:06am	6:22am	5:11am	12:43 pm	2:31am	5:42am	4:55pm	7:07pm	12:30am	4:40am	9:03am	4:09pm	11:29pm	03:04am	BCH Normal Ranges
Comprehensive Metabolic Panel																					
Sodium	135-145 mmol/L	134		136		136	139	137	137		137	132	142	145	145	148		148		142	135-138 mmol/L
Potassium	3.5-5.3 mmol/L	4.1		3.4		3.5	4	3.8	4.2		3.8	4.5	5.8	4.7	3.96	2.84		3.54		3.87	3.2-4.5 mmol/L
Cloride	97-100 mmol/L	98		100		101	103	103	102		99	97	102	107	101	101		102		102	96-109 mmol/L
CO2	24-32 mmol/L	26		26		25	21	26	27		30	22	24	11	9	17		30		28	22-30 mmol/L
Anion Gap	5 to 15	10		10		10	15	8	8		8	13	16	27	35	30		16		12	7-14 mmol/L
Glucose	70-99 mg/dL	91		128		98	71	129	85		99	108	233	69	168	228		178		136	70-99 mg/dL
Creatinine	0.60-1.30 mg/dL	<0.20		<0.20		<0.20	<0.20	<0.20	<0.20		<0.20	<0.20	0.32	<0.20	0.22	0.21		0.28		0.33	0.6-1.3 mg/dL
Calcium	8.7-10.7 mg/dL	9.1		9.5		8.3	8.4	7.8	7.6		7.7	7.6	14.2	6.7	8	6.9		8.2		7.7	8.4-10.5 mg/dL
Total Protein	6.0-8.0 g/dL	6.3		5.8		5.3	5	5	4.8		4.7	4.9	3.2		4.1		4.2	4.5		5.7	5.5-8.2 g/dL
Albumin	3.5-4.8	4.2		4		3.6	3.6	3.3	3.2		3.2	2.9	2		3.2		3.2	3.2		2.7	3.0-4.6 g/dL
Bilirubin, Total	0.3-1.2 mg/dL	0.4		0.4		0.4	0.3	0.2	0.2		0.1	0.3	0.3		0.4		0.4	0.3		0.5	0.3-1.2 mg/dL
Bilirubin, Direct	<=0.4 mg/dL			<0.1		0.1	0.1													<0.2	0.0-0.4mg/dL
Alk Phos	30-115 U/L	35		24		19	19	18	17		22	20	41		45		35	37		32	30-120 U/L
AST	10-40 U/L	36		31		53	62	57	50		80	140	143		485		300	330		308	2-40 U/L
ALT	10-40 U/L	40		37		43	48	48	41		49	63	58		150		96	85		52	3-30 U/L
Bun	7-23 mg/dL	9		6		11	18	9	15		13	16	16	14	18	19		18		22	7-18 mg/dL
eGFR	>=60 NA	133		133	122			131			130		>90								
Cystatin C	0.52=1.35 mg/L	0.64		0.64	0.77			0.66			0.67										
Magnesium	1.6-2.4 mg/dL	2.2		2		2.1	2.1	2.1	2.1		2.1	2			2.4	2	2.1	2.1		1.8	1.6-2.6 mg/dL
Phosphorus	2.5-4.5 mg/dL	4.1		4.9		3.5	3.4	2.7	2.9		3	3.4			5.4	5.6		7		6.2	2.7-4.9 mg/dL
GGT	7-50 U/L	20		15		14			13	16	15								15		12-55 U/L
CRP	<10.0 mg/L				1.1							1.9						2.68		6.65	<=0.50
Sed Rate	<15 mm/hr						3					5							3		
hsCRP	<1.0		0.4																		
Calcium Ionized	4.6-5.3 mg/dL							4.5			4.5										
Procalcitonin																				8.94	<=0.09
IgA	47 - 310 mg/dL	47																			
IgG	600 - 1640 mg/dL	381																			
LDH	110 - 240 U/L	262																1029			100-210U/L
Ferritin	23-336 ng/mL											5777								4281	10-320ng/mL

iSTAT Lactic Acid

CRD-TMH-001		D-30	D-1	D2		D3	D4	D5	D6				D7												D8					D9			
		8/31/2022	10/3/2022	10/5/2022		10/6/2022	10/7/2022	10/8/2022	10/9/2022				10/10/2022												10/11/2022					10/12/2022			
		10:08am	11:48am	8:16am	8:34am	9:36am	6:35am	5:21am	9:04am	12:02pm	5:47pm	8:56pm	12:09am	5:57am	11:19am	12:55	3:40pm	4:32pm	4:47pm	5:18pm	5:30pm	6:01pm	7:30pm	8:07pm	9:12pm	12:30am	1:30am	4:40am	8:24am	4:09pm	9:04pm	03:04am	8:18am	4:15pm	BCH Normal Ranges
iSTAT				Venous		Venous	Venous	Venous	Venous	Venous	Venous	Venous	Venous	Venous	Venous	Venous	Venous	Venous	Venous	Arterial	Venous	Venous	Arterial	Arterial	Arterial	Arterial	Arterial	Arterial	Arterial	Arterial	Venous	Venous + Arterial	Arterial	Arterial	
pH, POCT	7.31-7.41 pH			7.45		7.41	7.22	7.28	7.46	7.45	7.41	7.43	7.43	7.43	7.41	7.44	7.4	7.02	7.33		7.09	7.51									7.464	7.353			7.31-7.41 pH
pCO2, POCT	41-51 mm Hg			36.4		40.2	59.4	58.8	41.5	40.9	41	36.2	35.7	33.5	32.4	30.3	33.4	113.4	50.2		87.7	23.8									45.5	55.8			35-45 mm Hg
pO2, POCT	35-40 mm Hg			41		42	56	45	36	32	32	32	29	29	29	31	30	53	73		<15	>530									36	64.7			30-50 mm Hg
Base Excess, POCT	0-3			2		1	-3	1	5	4	1	0	0	-2	-4	-4	-6	-2	0		-3	-4													
HCO3, POCT	23-28 mmol/L			25.6		25.3	24.5	27.7	29.3	28.3	25.9	24.2	23.8	22	20.3	20.4	20.5	29.1	26.2		26.7	18.8									33	31			20-33 mmol/L
TCO2, POCT	24-29 mmol/L			27		27	26	29	31	30	27	25	25	23	21	21	22	33	28		29	19													
Saturated O2, POCT	70-75 %			79		78	82	74	71	65	61	63	58	57	57	63	59	66	93												72	92			60-85 %
Lactate, POCT	0.9-1.7 mmol/L			0.64		0.68	0.63	0.65	0.65	0.94	1.09	1.38	0.94	1.1	1.19	1.2	1.28	11.32			13.69	14.56	15.42	17.9	16.17	15.11										
pH, POCT	7.35-7.45 pH																			7.41			7.33	7.36	7.45	7.396	7.754	7.461	7.41	7.44		7.361	7.384	7.432	7.35-7.45 pH
pCO2, POCT	35-45 mm Hg																			21.2			22.9	22.7	25.3	15	22.8	26	45.7	48.3		54	32.4	45.8	35-45 mm Hg
pO2, POCT	80-105 mm Hg																			103			511	497	522	577	411	269	246	129		244	136	115	85-105 mm Hg
Base Excess, POCT	0-3																			-11			-14	-13	-7										
HCO3, POCT	21-28 mmol/L																			13.4			12.2	12.7	17.4	9	32	18	29	33		30	31	30	24-27 mmol/L
TCO2, POCT	23-27 mmol/L																			14			13	13	18										
Saturated O2, POCT	95-98 %																			98			100	100	100	100	>100	100	100	100		100	99	99	94-98 %
Lactic Acid	0.3-1.9 mmol/L	1.3	1		0.7														11.8							15	15	8.7	4.6	3.6	4.2	3.5	4	3.3	0.3-1.9 mmol/L

Other Labs

CRD-TMH-001		D-30	D-14	D-7	D-1	D2	D3			D4		D5		D6				D7				D8			D9			
		8/31/2022	9/20/2022	9/28/2022	10/3/2022	10/5/2022	10/6/2022			10/7/2022		10/8/2022		10/9/2022				10/10/2022				10/11/2022			10/12/2022			
	Umass Normal Ranges	10:08am	10:26am	9:12am	11:48am	8:42am	9:06am	10:57am	5:57pm	6:22am	8:45am	5:11am	12:43pm	2:31am	11:34am	12:14pm	5:42pm	12:16am	5:42am	12:56pm	2:44pm	4:55pm	4:40am	4:09pm	9:04pm	3:04am	4:01am	8:18am	4:15pm	BCH Normal Ranges
Troponin I	0.01-0.04 ng/mL	<0.01			<0.01	0.01	0.03		0.01	0.01		0.03		0.59		1.37	2.55	1.81	1.41	1.52		4.44							
Troponin T																							1.94		1.65				
BNP	<100 pg/mL	15			11	53		94	114		125	81		246		461	1011	1122	1576		1578		2058		4181				0-100 pg/mL
NT PROBNP		67			63	240					556			1780															
CK	49-348 U/L	949			858	1413							3042																
CK-MB	<4.4 ng/mL	20.1			19	38.5						13.7	41.1																
C3	82-185 mg/dL	143							106						3861				6507										
C4	15-53 mg/dL	19							15						34				60.4										
Complement, Total	31-60 U/mL																				<10								
SC5b-9	<=244 ng/mL										453																		
Haptoglobin	43-212 mg/dL				134	146								224															
interferon gamma	<4.2 pg/mL																<4.2												
interleukin-1 beta	<= 6.7 pg/mL																<6.5												
interleukin-6	<=2.0 pg/mL																2.8												
interleukin-10	<=2.8 pg/mL																7.3												
Tumor Necrosis FCTR Alpha	0.56-1.40 pg/mL													<0.16															
PT	9.6-12.4 seconds	10.9																							17.2	15.6	16.3	16.7	16.4	17.7	12.1-14.6
PTT	23.0-32.0 seconds	25																							>200	103.5	80.7	59.2	45.1	41.4	25.0-37.0
Fibrinogen	150-440 mg/dL	363																							236	291	254	260	275	264	200-400mg/dl
D-Dimer	<0.50 mg/L FEU	0.53																													
Anti-AAV9	<1:25	<1:25	<1:25	<1:25	<1:25	<1:25	<1:25																								
Adenovirus Antibody	<1:8													<1:8																
EBV Viral Cap IgM	<36.00=neg										<36.00																				
EBV Viral Cap IgG	<18.00=neg										<18.00																				
EBV Nuclear Ag Ab	<18.00=neg										<18.00																				
CMV IgM	<30.00=neg										<30.00																				
CMV IgG	<60.0=nega										<60.0																				

Pericardial Fluid

CRD-TMH-001		D4
		10/7/2022
	Umass Normal Ranges	8:45am
PERICARDIAL FLUID		
LDH PC Fluid	U/L	386
pH PC Fluid		<7.70
Glucose PC Fluid	mg/dL	95
Protein PC Fluid	g/dL	3.4
Cell Count PC Fluid		118
Color PC Fluid		hazy,yellow
STAT Gram Stain		1+ mononuclear cells, no organisms
Mycobacteria Culture		no growth to date
Fungas Culture		no fungal growth at 4 weeks
Body Fluid Culture		Staphyloccus Lugdunensis
Flow Cytometry		polytypic B cells and immunophenotypically normal T cells are detected. The CD4:CD8 ration is reversed at 0.56:1. No immature myelomonocytic cells are detected.

Urine

CRD-TMH-001		D-30	D1	D2	D3	D4	D5
		8/31/2022	10/4/2022	10/5/2022	10/6/2022	10/7/2022	10/8/2022
	Umass Normal Ranges	10:08am	08:56am	8:42am	9:06am	6:22am	5:11am
Urine Osmolality	70-900 mOsm/kg	201	516	423	164	356	
Microalbumin urine	mg/dl		1.1	<1.0	<1.0	<1.0	
Creatinine Urine	24-392 mg/dL		34	16	3	7	
Micro/Creat Ratio	<30 mcg/mg		32.4			<1.0mcg/mgCr	
Protein Urine	mg/dL		9	7	<4	<4	
Protein/Creat Ratio			265	438		unable to calc	
Urinalysis							
color, urine	yellow, light, clear		yellow	light yellow	yellow	light yellow	yellow
clarity, urine	clear		slightly cloudy	clear	slightly cloudy	clear	slightly cloudy
spec. grav. Urine	1.005 - 1.030		1.018	1.013	1.03	1.008	1.024
pH, urine	4.6 - 8.0		6	6	6	6	6
protein, urine	negative		negative	negative	1+	negative	1+
glucose, urine	negative		negative	negative	negative	negative	negative
ketones, urine	negative		1+	negative	trace	negative	2+
bilirubin, urine	negative		negative	negative	negative	negative	negative
blood, urine	negative		negative	negative	negative	negative	negative
nitrite, urine	negative		negative	negative	negative	negative	negative
urobilinigen, urine	normal		normal	normal	normal	normal	normal
leukocyte esterase, urine			negative	negative	negative	negative	negative
WBC, urine			5		1		
RBC, urine			0		0		
hyaline casts, urine			0		0		
bacteria, urine			none		occasional		
mucus, urine			many		few		

Immunology

CRD-TMH-001	D8	
	10/11/2022	
	4:09pm	BCH Normal Ranges
TEMRA CD4+CD45RA5CCR7-T cells	**0.4**	0.1-4.0 %
Naïve CD4+CD45RA+CCR7+T cells	**75.4**	21.0-61.4 %
Effector memory CD4+CD45TA-CCR7-T cells	**5.7**	7.6-25.1 %
Central memory CD4+CD45RA-CCR7+T cells	**18.5**	26.8-62.1 %
TEMRA CD8+CD45RA+CCR7-T cells	**3.7**	5.6-43.9 %
Naïve CD8+CD45RA+CCR7+T cells	**83.8**	11.4-66.5 %
Effector memory CD8+CD45RA-CCR7-T cells	**7.9**	16.8-54.6 %
Central memory CD8+CD45RA-CCR7+T cells	4.6	3.7-23.2 %
Switched memory IgD-CD27+B cells	NR	8.30-27.80
Unswitched memory IgD+CD27+B cells	NR	7.00-23.80
Naïve IgD+CD27-B cells	NR	48.40-79.70
*not reportable; Naïve IgD+CD27=B cells, switched memory IgD-CD27+B cells, unswitched memory IgD+CD27+B cells. The patient is lymphopenic. The number of lymphocytes acquired are insufficient for analysis. Results cannot be reported.		