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Supplemental Information

Protease-Activatable Adeno-Associated Virus Vector for Gene Delivery to Damaged Heart Tissue

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SUPPLEMENTAL FIGURES

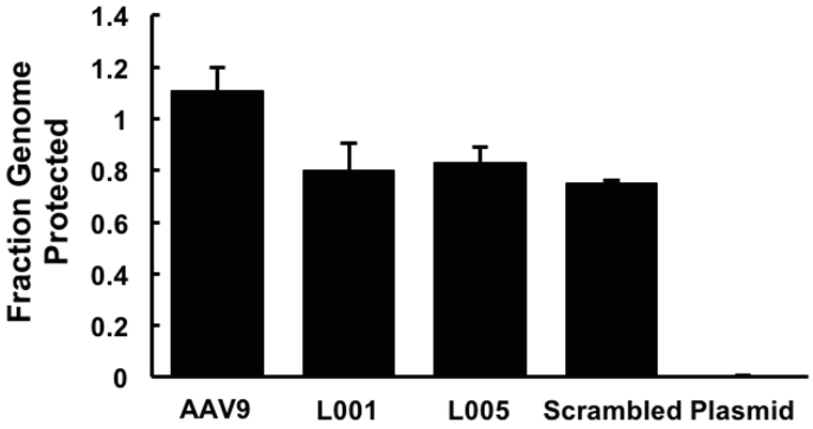

Figure S1: Structural characterization of provectors. Provectors display genome protection ability similar to AAV9. Vectors were incubated with benzonase nuclease and fraction of genomes protected was quantified with qPCR. Unprotected plasmid DNA served as control. Error bars: SEM, N=3.

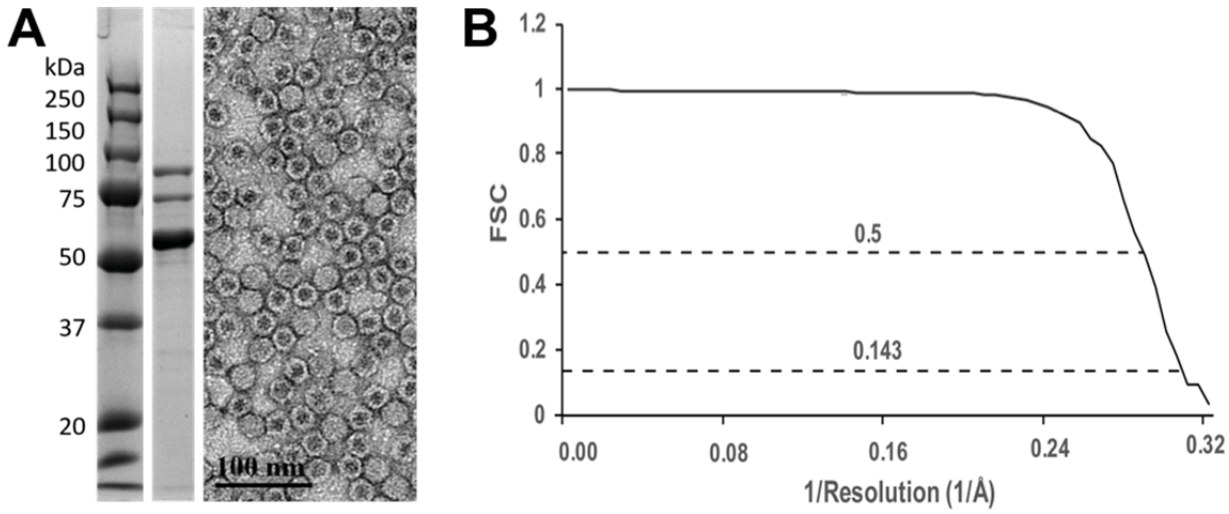

Figure S2: Production and structure determination of L001. (**A**) SDS PAGE (left) showing the molecular marker and the purified vector with VP1:VP2:VP3 in a 1:1:10 ratio and the negative stain EM (right) of purified vector. (**B**) To determine the resolution of the map, the Fourier Shell Correlation versus the inverse of the resolution from the last refinement cycle was plotted. The resolution for the map at FSC=0.143 is 3.16Å.

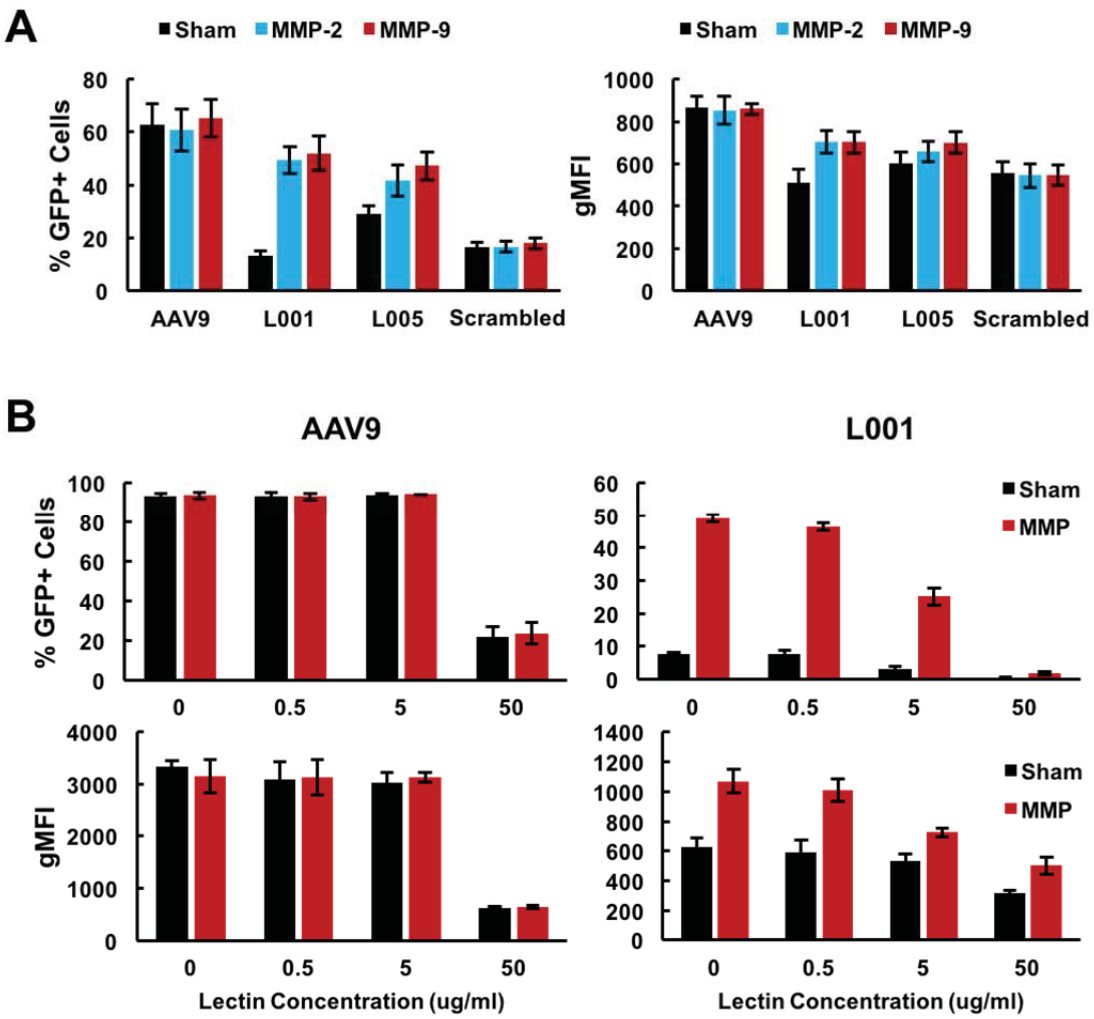

Figure S3: Transduction data corresponding to transduction index (TI) calculation. (**A**) % GFP+ cells (left) and geometric mean fluorescence intensity (right) quantified via flow cytometry used to calculate TI of Fig. 3B. Error bars: SEM, N=3. (B) % GFP+ cells (top) and geometric mean fluorescence intensity (bottom) quantified via flow cytometry used to calculate TI of Fig. 3C. Error bars: SEM, N=2.

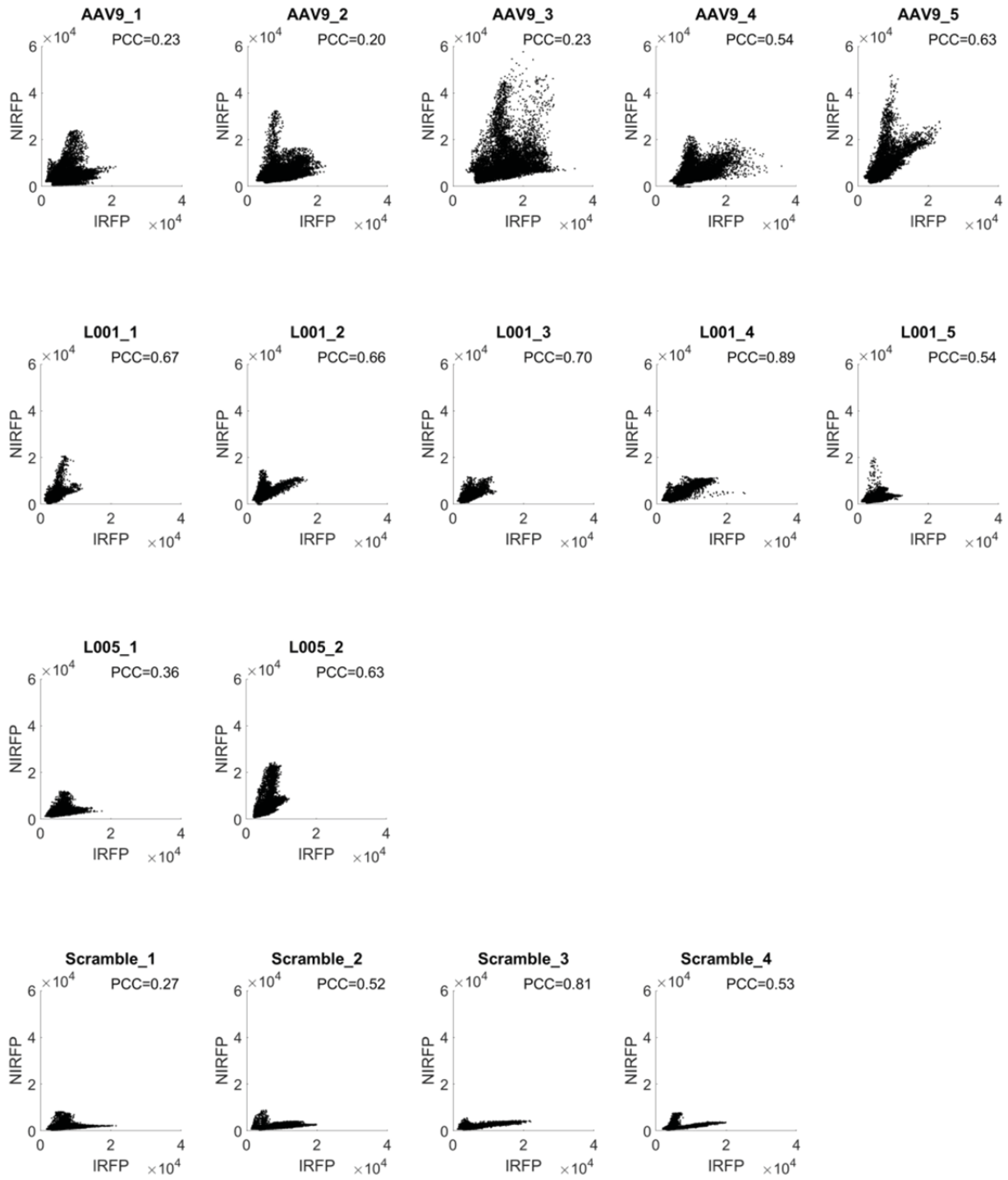

Figure S4: Scatter plots of iRFP and NIRF pixel intensities of heart slices. NIRF pixel intensities are plotted against IRFP pixel intensities to show the correlation between the two signals, and the resulting Pearson's Correlation Coefficient (PCC) is indicated. A higher correlation signifies a higher level of co-localization, indicating the specificity of the virus's ability to target tissue with elevated MMP expression.

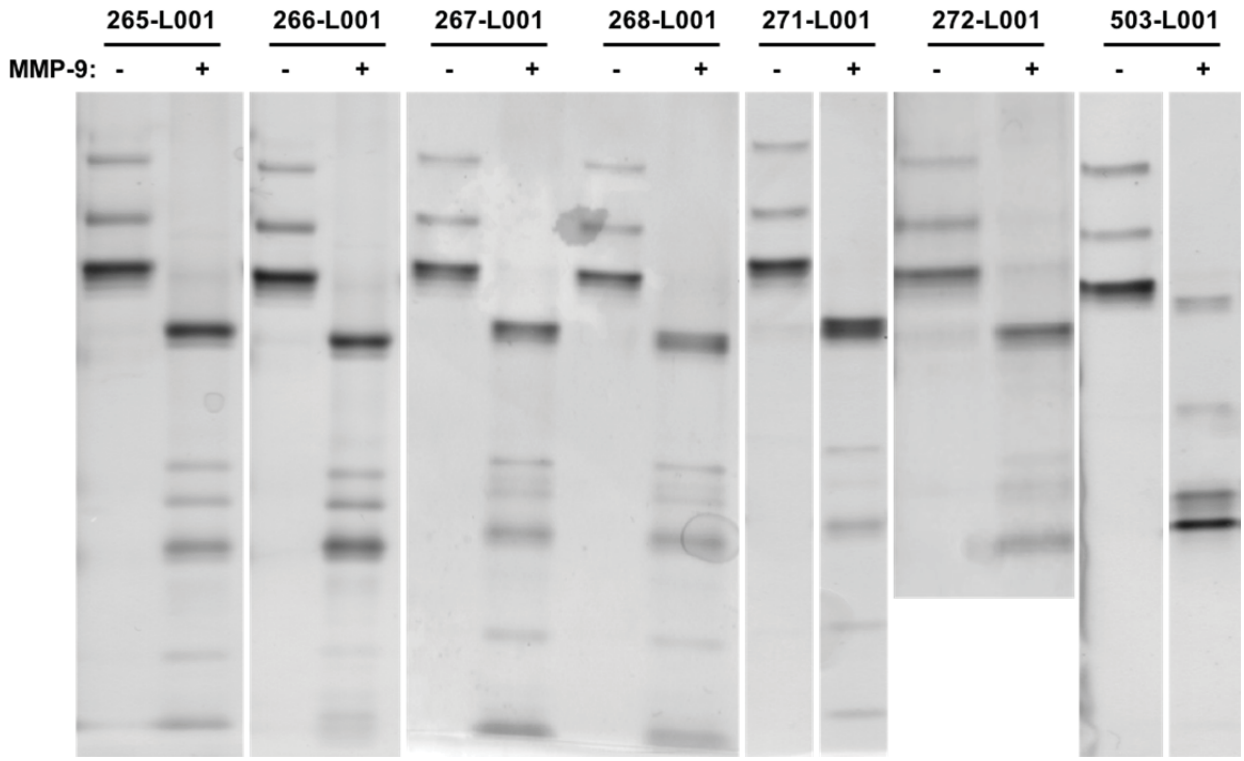

Figure S5: Failed lock insertion site variants are susceptible to MMP cleavage. Proteolytic cleavage fragments of the indicated variant capsid subunits can be detected via silver stain. Vectors were treated with MMP-9 or sham buffer. Intact VPs are observed in the sham conditions, while N-terminal and C-terminal fragments are observed for all variants following treatment with MMP-9.

SUPPLEMENTAL TABLES

Table S1: Lock insertion sites tested

Insert Location[a]	Titer[b]	MMP Cleavable[c]	Switchable Transduction[d]
S265	+	Y	N
G266	+	Y	N
G267	+	Y	N
S268	+	Y	N
D271	+	Y	N
N272	+	Y	N
G453	++	Y	Y
S454	-	ND	ND
N470	-	ND	ND
A472	-	ND	ND
V473	-	ND	ND
W503	+	Y	N

[a] Locks inserted after indicated residue. Residues previously identified as being part of the galactose binding pocket are in bold.[25]

[b] Titer relative to WT AAV9: ++, within 1 log of AAV9; +, < 1 log of AAV9; -, < 2 log of AAV9, too low for further characterization.

[c] Capsid VP cleavage determined by visualization on silver stain.

[d] Tested on CHO-Lec2 cells at MOI of 5000. Y: Yes, N: No, ND: Not determined.

Table S2: Summary of data collection parameters and refinement statistics

Data Collection Parameters	
Total number of micrographs	1444
Defocus range (μm)	0.8-3.91
Electron dose(e^- / $Å^2$)	59.40
Frames / micrograph	30
Pixel size (Å / pixel)	0.974
Starting number of particles	145614
Particles used for final map	114044
B-factor used for final map ($Å^2$)	50
Resolution of final map (Å)	3.16
PHENIX model Refinement Statistics	
Residue range	219-456 and 481-762
Map CC	0.8477
RMSD [bonds] (Å)	0.010
RMSD [angles] (Å)	0.758
All-atom clashscore	0.88
Ramachandran plot	
Favored (%)	95.53
Allowed (%)	4.07
Outliers (%)	0.00
Rotamer outliers (%)	0.00
C-β deviations	0.00

Table S3: Peptide lock oligo sequences

Lock Name	Oligo Name	DNA Sequence	Peptide Lock Sequence
L001	L001 FW	CCGGCGTGCCTATGAGTATGAGAGG AGGAGGAGATGATGATGATGGAGTG CCTATGAGTATGAGAGGCGGAGG	AG-VPMSMRGG-G-DDDD-G-VPMSMRGG-GA
	L001 RV	GCGCCTCCGCCTCTCATACTCATAG GCACTCCATCATCATCATCTCCTCCT CCTCTCATACTCATAGGCACG	
L005	L005 FW	CCGGCCCTCTGGGACTCGCCAGAGG TGATGATGACGACGGACCACTGGGA CTGGCACGTG	AG-PLGLAR-G-DDDD-G-PLGLAR-GA
	L005 RV	GCGCCACGTGCCAGTCCCAGTGGTC CGTCGTCATCATCACCTCTGGCGAG TCCCAGAGGG	
Scrambled	L001-S1 FW	CCGGCAGTATGGTGGGAATGAGACC TGGAGGAGATGATGATGATGGAAGT ATGGTGGGAATGAGACCTGGAG	AG-SMVGMRPG-G-DDDD-G-SMVGMRPG-GA
	L001-S1 RV	GCGCCTCCAGGTCTCATTCCCACCA TACTTCCATCATCATCATCTCCTCCA GGTCTCATTCCCACCATACTG	

Table S4: qPCR Primers

Transgene	Primer Name	Sequence
CMV	CMV FW	TCACGGGGATTTCCAAGTCTC
	CMV RV	AATGGGGCGGAGTTGTTACGAC
iRFP	iRFP3 FW	AAGTGATCGCAGAGGATCGG
	iRFP3 RV	CACGGTTGAGGCAGGATAGT
18s	18s1 FW	AACCCGTTGAACCCCATT
	18s1 RV	CCATCCAATCGGTAGTAGCG