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Supplemental Information

AAV capsid engineering identified two novel variants with improved *in vivo* tropism for cardiomyocytes

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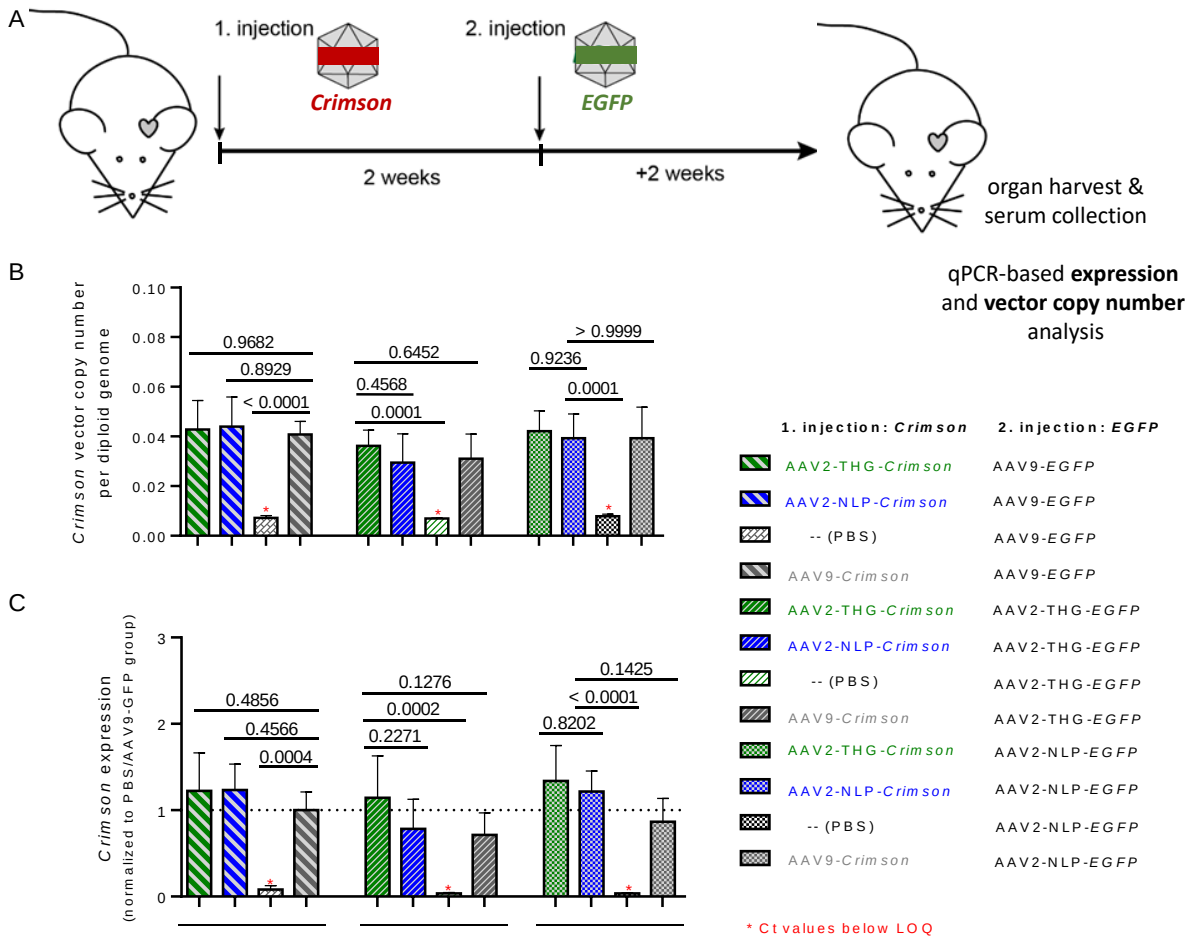

Supplemental Fig. 1: *Crimson* vector copy number and expression analysis.

(A) AAV vectors delivering *GFP* were injected two weeks after a previous AAV vector injection of different AAV variants delivering the *Crimson* transgene ($4x10^{10}$ vector genomes or PBS as control). (B+C) Overview of vector copy number analysis (B) and expression analysis (C) from the first injected AAV vector (*Crimson* transgene) showing comparable vector copy numbers and expression levels, respectively, in all groups receiving the *Crimson* transgene by AAV-mediated delivery. n=5, data are means ± SD. P-values were determined by one-way ANOVA with Dunnett's multiple comparison to the group which received the same AAV capsid variant (AAV2-THG, AAV2-NLP, AAV9) in the first and second injection. (LOQ: limit of quantification)

Supplemental Table 1: Echocardiographic analysis of sham and TAC mice after therapeutic overexpression of lncRNA *H19* using AAV9, AAV2-THGTPAD and AAV2-NLPGSGD.

	sham	**TAC**			
	AAV9-empty	**AAV9-empty**	**AAV9-H19**	**AAV2-THG-H19**	**AAV2-NLP-H19**
LV ejection fraction (%)	59.2 ± 4.8	31.5 ± 9.7	35.6 ± 8	54.2 ± 5.6 ***	44.7 ± 11.1 *
Fractional shortening (%)	31.2 ± 3.4	15.1 ± 5.1	17.2 ± 4	28.1 ± 3.5 ***	22.5 ± 6.4 *
Cardiac output (µl/min)	20.9 ± 5	18.5 ± 5.5	18.3 ± 7.4	24.7 ± 6.9	23.8 ± 7.4
LV mass (mg)	89.8 ± 18.8	199.6 ± 48.7	167.7 ± 44.1	130.4 ± 46.5 **	147.3 ± 52.4 **
End-diastolic volume (µl)	77.6 ± 10.7	130.7 ± 38.8	117.9 ± 37.6	96.2 ± 26.7	112.6 ± 38.3
End-systolic volume (µl)	31.6 ± 5.4	92.2 ± 38.2	78.1 ± 35.9	45.1 ± 17 **	64.9 ± 33.5
Stroke volume (µl)	46 ± 7.8	38.5 ± 8.3	39.8 ± 8.9	51.1 ± 10.4 **	47.7 ± 11.4
End-diastolic diameter (mm)	4.2 ± 0.2	5.2 ± 0.7	4.9 ± 0.6	4.5 ± 0.5 *	4.8 ± 0.7
End-systolic diameter (mm)	2.9 ± 0.2	4.4 ± 0.8	4.1 ± 0.7	3.3 ± 0.5 ***	3.8 ± 0.8
End-diastolic anterior wall (mm)	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.7 ± 0.1 **	0.8 ± 0.1 *
End-systolic anterior wall (mm)	0.9 ± 0.1	1 ± 0.1	1 ± 0.1	1 ± 0.2	1 ± 0.2
End-diastolic posterior wall (mm)	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1 *
End-systolic posterior wall (mm)	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1 ± 0.1	0.9 ± 0.1
End-diastolic IV septum (mm)	0.6 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.8 ± 0.1 **	0.7 ± 0.1 **
End-systolic IV septum (mm)	0.8 ± 0.1	1 ± 0.2	1 ± 0.1	1 ± 0.2	1 ± 0.1
End-diastolic LV internal diameter (mm)	4.2 ± 0.2	5.2 ± 0.7	5 ± 0.6	4.5 ± 0.6 *	4.8 ± 0.7
End-systolic LV internal diameter (mm)	2.9 ± 0.2	4.4 ± 0.8	4.1 ± 0.7	3.3 ± 0.5 **	3.8 ± 0.8
Heart rate (bpm)	452.2 ± 59.8	479.2 ± 83.9	450 ± 93.5	479.5 ± 76.5	498.6 ± 82.4

Data are mean ± SD

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ between *H19* overexpression with the respective AAV vector variant (AAV9 or AAV2-THGTPAD or AAV2- NLPGSGD) and TAC AAV9-empty; one-way ANOVA with Dunnett's multiple comparison to TAC AAV9-empty; IV – interventricular, LV – left ventricle