

Supporting Information

Identification of new AAV vectors with enhanced blood-brain barrier penetration efficiency via organ-on-a-chip

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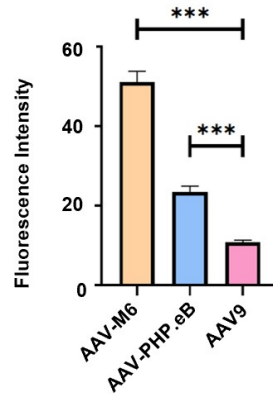


Fig.S1 Quantitative analysis of AAV fluorescence in the HA-1800 cell layer.

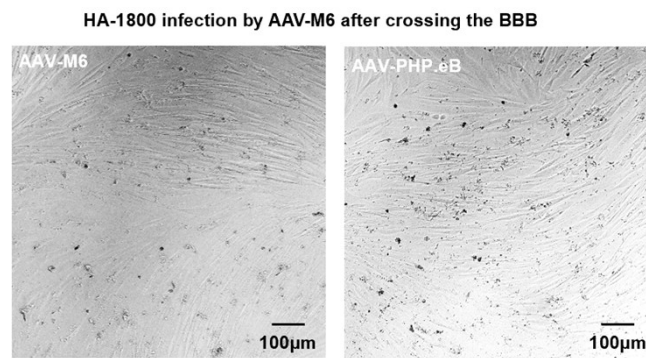


Fig.S2 Bright field images of HA-1800 cells transfected with AAV-M6 or AAV.PHP.eB.

Scale bar: 100 µm.

Table S1 Primers used in this study.

Name of primers	The primer sequences (5'->3')
Cap-F	CATCTTTGAACAATAAATGATTTAAATCAGGTATG
Cap-R	AAAGTTCAACTGAAACGAATCAACC
AAV-M6-F	CAAGCGATGCCTCGGACGCCGGTGCACAGGCGCAGACCGGCTG
AAV-M6-R	CGGCGTCCGAGGCATCGCTTGGGCACTCTGGTGGTTTGTGGCCAC
AAV-M8-F	CAAACGATGCCTAGGCATCCGCCGGCACAGGCGCAGACCGGCTG
AAV-M8-R	GCGGATGCCTAGGCATCGTTTGGGCACTCTGGTGGTTTGTGGCCACT

Table S2 Amino acid sequences of the AAV-M6 and AAV-M8 capsid proteins and heptapeptide insertions.

AAV variant	Amino acid sequences
AAV-M6 (peptide)	AMPRTPG
AAV-M8 (peptide)	TMPRHPP
AAV-M6 (variant)	MAADGYLPDWLEDNLSEGIREW WALKPGAPQPKANQQHQDNARGLVLP G YKYLGPNGLDKGE PVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADA E F QERLKEDTSFGGNLGRAVFQAKKRLLLEPLGLVEEAAKTAPGKKRPVEQSPQ E P DSSAGIGKSGAQPAAKRLNFGQTGDTEVPDPQPIGEPPAAPSGVGS L T M A S G G G A P V A D N N E G A D G V G S S S G N W H C D S Q W L G D R V I T T S T R T W A L P T Y N N H L Y K Q I S N S T S G G S S N D N A Y F G Y S T P W G Y F D F N R F H C H F S P R D W Q R L I N N N W G F R P K R L N F K L F N I Q V K E V T D N N G V K T I A N N L T S T V Q V F T D S D Y Q L P Y V L G S A H E G L P P P A D V F M I P Q Y G Y L T L N D G S Q A V G R S S F Y C L E Y F P S Q M L R T G N N F Q F S Y E F E N V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y Y L S K T I N G S G Q N Q Q T L K F S V A G P S N M A V Q G R N Y I P G P S Y R Q Q R V S T T V T Q N N N S E F A W P G A S S W A L N G R N S L M N P G P A M A S H K E G E D R F F L S G S L I F G K Q G T G R D N V D A D K V M I T N E E E I K T T N P V A T E S Y G Q V A T N H Q S A Q A M P R T P G A Q A Q T G W V Q N Q G I L P G M V W Q D R D V Y L Q G P I W A K I P H T D G N F H P S P L M G G F G M K H P P P Q I L I K N T P V P A D P P T A F N K D K L N S F I T Q Y S T G Q V S V E I E W E L Q K E N S K R W N P E I Q Y T S N Y Y K S N N V E F A V N T E G V Y S E P R P I G T R Y L T R N L *
AAV-M8 (variant)	MAADGYLPDWLEDNLSEGIREW WALKPGAPQPKANQQHQDNARGLVLP G YKYLGPNGLDKGE PVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADA E F QERLKEDTSFGGNLGRAVFQAKKRLLLEPLGLVEEAAKTAPGKKRPVEQSPQ E P DSSAGIGKSGAQPAAKRLNFGQTGDTEVPDPQPIGEPPAAPSGVGS L T M A S G G G A P V A D N N E G A D G V G S S S G N W H C D S Q W L G D R V I T T S T R T W A L P T Y N N H L Y K Q I S N S T S G G S S N D N A Y F G Y S T P W G Y F D F N R F H C H F S P R D W Q R L I N N N W G F R P K R L N F K L F N I Q V K E V T D N N G V K T I A N N L T S T V Q V F T D S D Y Q L P Y V L G S A H E G L P P P A D V F M I P Q Y G Y L T L N D G S Q A V G R S S F Y C L E Y F P S Q M L R T G N N F Q F S Y E F E N V P F H S S Y A H S Q S L D R L M N P L I D Q Y L Y Y L S K T I N G S G Q N Q Q T L K F S V A G P S N M A V Q G R N Y I P G P S Y R Q Q R V S T T V T Q N N N S E F A W P G A S S W A L N G R N S L M N P G P A M A S H K E G E D R F F L S G S L I F G K Q G T G R D N V D A D K V M I T N E E E I K T T N P V A T E S Y G Q V A T N H Q S A Q T M P R H P P A Q A Q T G W V Q N Q G I L P G M V W Q D R D V Y L Q G P I W A K I P H T D G N F H P S P L M G G F G M K H P P P Q I L I K N T P V P A D P P T A F N K D K L N S F I T Q Y S T G Q V S V E I E W E L Q K E N S K R W N P E I Q Y T S N Y Y K S N N V E F A V N T E G V Y S E P R P I G T R Y L T R N L *

Table S3 Nucleotide sequences of heterologous peptides and AAV capsid protein mutants.

AAV variant	Nucleotide sequences
AAV-M6 (peptide)	GCGATGCCTCGGACGCCGGT
AAV-M8 (peptide)	ACGATGCCTAGGCATCCGCCG
AAV-M6 (variant)	<p>ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTTAGT GAAGGAATTCGCGAGTGGTGGGCTTTGAAACCTGGAGCCCCTCAACCC AAGGCAAATCAACAACATCAAGACAACGCTCGAGGTCTTGTGCTTCCG GGTTACAAATACCTTGGACCCGGCAACGGACTCGACAAGGGGGAGCC GGTCAACGCAGCAGACGCGGCGGCCCTCGAGCACGACAAGGCCTACG ACCAGCAGCTCAAGGCCGGAGACAACCCGTACCTCAAGTACAACCACG CCGACGCCGAGTTCAGGAGCGGCTCAAAGAAGATACGTCTTTTGGG GGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAAAGAGGCTTCTTGAA CCTCTTGGTCTGGTTGAGGAAGCGGCTAAGACGGCTCCTGGAAAGAA GAGGCCTGTAGAGCAGTCTCCTCAGGAACCGGACTCCTCCGCGGGTAT TGGCAAATCGGGTGACAGCCCGCTAAAAGAGACTCAATTCGGTCA GACTGGCGACACAGAGTCAGTCCCAGACCCTCAACCAATCGGAGAACC TCCCGCAGCCCCCTCAGGTGTGGGATCTTTACAATGGCTTCAGGTGG TGGCGCACCAAGTGGCAGACAATAACGAAGGTGCCGATGGAGTGGGTA GTTCTCGGGAAATTGGCATTGCGATTCCAATGGCTGGGGGACAGA GTCATCACCACCAGCACCCGAACCTGGGCCCTGCCACCTACAACAATC ACCTCTACAAGCAAATCTCCAACAGCACATCTGGAGGATCTTCAAATGA CAACGCCTACTTCGGCTACAGCACCCCTGGGGGTATTTTGACTTCAAC AGATTCCACTGCCACTTCTCACCACGTGACTGGCAGCGACTCATCAACA ACAACCTGGGGATTCCGGCCTAAGCGACTCAACTCAAGCTCTTCAACAT TCAGGTCAAAGAGGTTACGGACAACAATGGAGTCAAGACCATCGCCA ATAACCTTACCAGCACGGTCCAGGTCTTACGGACTCAGACTATCAGCT CCCGTACGTGCTCGGGTCGGCTCACGAGGGTGCCTCCCGCCGTTCC AGCGGACGTTTTTCATGATTCTCAGTACGGGTATCTGACGCTTAATGAT GGAAGCCAGGCCGTGGGTCGTTTCGTCCTTTTACTGCCTGGAATATTTCC CGTCGCAAATGCTAAGAACGGGTAACAACCTCCAGTTCAGCTACGAGT TTGAGAACGTACCTTTCCATAGCAGCTACGCTCACAGCCAAAGCCTGG ACCGACTAATGAATCCACTCATCGACCAATACTTGTAATCTCTCTCAA GACTATTAACGGTTCTGGACAGAATCAACAAACGCTAAAATTCAAGTGT GGCCGGACCCAGCAACATGGCTGTCCAGGGAAGAACTACATACCTG GACCCAGCTACCGACAACAACGTGTCTCAACCACTGTGACTCAAACA ACAACAGCGAATTTGCTTGGCCTGGAGCTTCTTCTTGGGCTCTCAATGG ACGTAATAGCTTGATGAATCCTGGACCTGCTATGGCCAGCCACAAAGA AGGAGAGGACCGTTTCTTCTTTGTCTGGATCTTTAATTTTTGGCAA CAAGGAACTGGAAGAGACAACGTGGATGCGGACAAAGTCATGATAAC CAACGAAGAAGAAATTAATACTACTAACCCTGGTAGCAACGGAGTCCTA TGGACAAGTGGCCACAACCACCAGAGTGCCCAAGCGATGCCTCGGA</p>

CGCCGGGTGCACAGGCGCAGACCGGCTGGGTTCAAACCAAGGAATA
CTTCCGGGTATGGTTTGGCAGGACAGAGATGTGTACCTGCAAGGACCC
ATTTGGGCCAAAATTCTCACACGGACGGCAACTTTCACCCCTTCTCCGC
TGATGGGAGGGTTTGAATGAAGCACCCGCCTCCTCAGATCCTCATCA
AAAACACACCTGTACCTGCGGATCCTCCAACGGCCTTCAACAAGGACA
AGCTGAACTCTTTCATCACCCAGTATTCTACTGGCCAAGTCAGCGTGA
GATCGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCG
GAGATCCAGTACACTTCCAATTACAAGTCTAATAATGTTGAATTTG
CTGTTAATACTGAAGGTGTATATAGTGAACCCCGCCCCATTGGCACCA
GATACCTGACTCGTAATCTGTAA

AAV-M8
(variant)

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTTAGT
GAAGGAATTCGCGAGTGGTGGGCTTTGAAACCTGGAGCCCCTCAACCC
AAGGCAAATCAACAACATCAAGACAACGCTCGAGGTCTTGTGCTTCCG
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GGTCAACGCAGCAGACGCGGCGGCCCTCGAGCACGACAAGGCCTACG
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TGGCAAATCGGGTGCACAGCCCGCTAAAAGAGACTCAATTTCCGGTCA
GACTGGCGACACAGAGTCAGTCCCAGACCCTCAACCAATCGGAGAACC
TCCCGCAGCCCCCTCAGGTGTGGGATCTCTTACAATGGCTTCAGGTGG
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GTCATCACCACCAGCACCCGAACCTGGGCCCTGCCACCTACAACAATC
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GACTATTAACGGTTCTGGACAGAATCAACAAACGCTAAAATTCAGTGT
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CAAGGAACTGGAAGAGACAACGTGGATGCGGACAAAGTCATGATAAC
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 ATTTGGGCCAAAATTCCTCACACGGACGGCAACTTTCACCCTTCTCCGC
 TGATGGGAGGGTTTGAATGAAGCACCCGCCTCCTCAGATCCTCATCA
 AAAACACACCTGTACCTGCGGATCCTCCAACGGCCTTCAACAAGGACA
 AGCTGAACTCTTTCATCACCCAGTATTCTACTGGCCAAAGTCAGCGTGGA
 GATCGAGTGGGAGCTGCAGAAGGAAAACAGCAAGCGCTGGAACCCG
 GAGATCCAGTACACTTCCAATAATTACAAGTCTAATAATGTTGAATTTG
 CTGTTAATACTGAAGGTGTATATAGTGAACCCCGCCCCATTGGCACCA
 GATACCTGACTCGTAATCTGTAA

Table S4 Complete list of interacting residues and binding constant K_i from molecular docking simulation of human virus receptor proteins with heptapeptide insertion residues.

	Protein	Peptide	Binding Constant K_i
LY6D	THR-104	THR-5	0.96 μ M
	THR-102	THR-5	
	HIS97	ARG4	
	THR47	ARG4	
	GLN85	ARG4	
	LYS95	ARG4	
LY6E	ARG108	THR-6	13.52 μ M
	SER-101	ARG-5	
M6PR	ASP-129	ARG-4	0.12 μ M
	GLU-127	ARG-4	
	THR-101	ARG-4	
	ARG105	ARG-4	
	THR-85	ARG-4	
	GLY-98	ALA-1	
MELTF	ASN141	GLY-7	23.52 μ M
	THR-137	THR-5	
	TYR-210	PRO-3	
HBEFG	ARG-142	GLY-7	45.10 μ M