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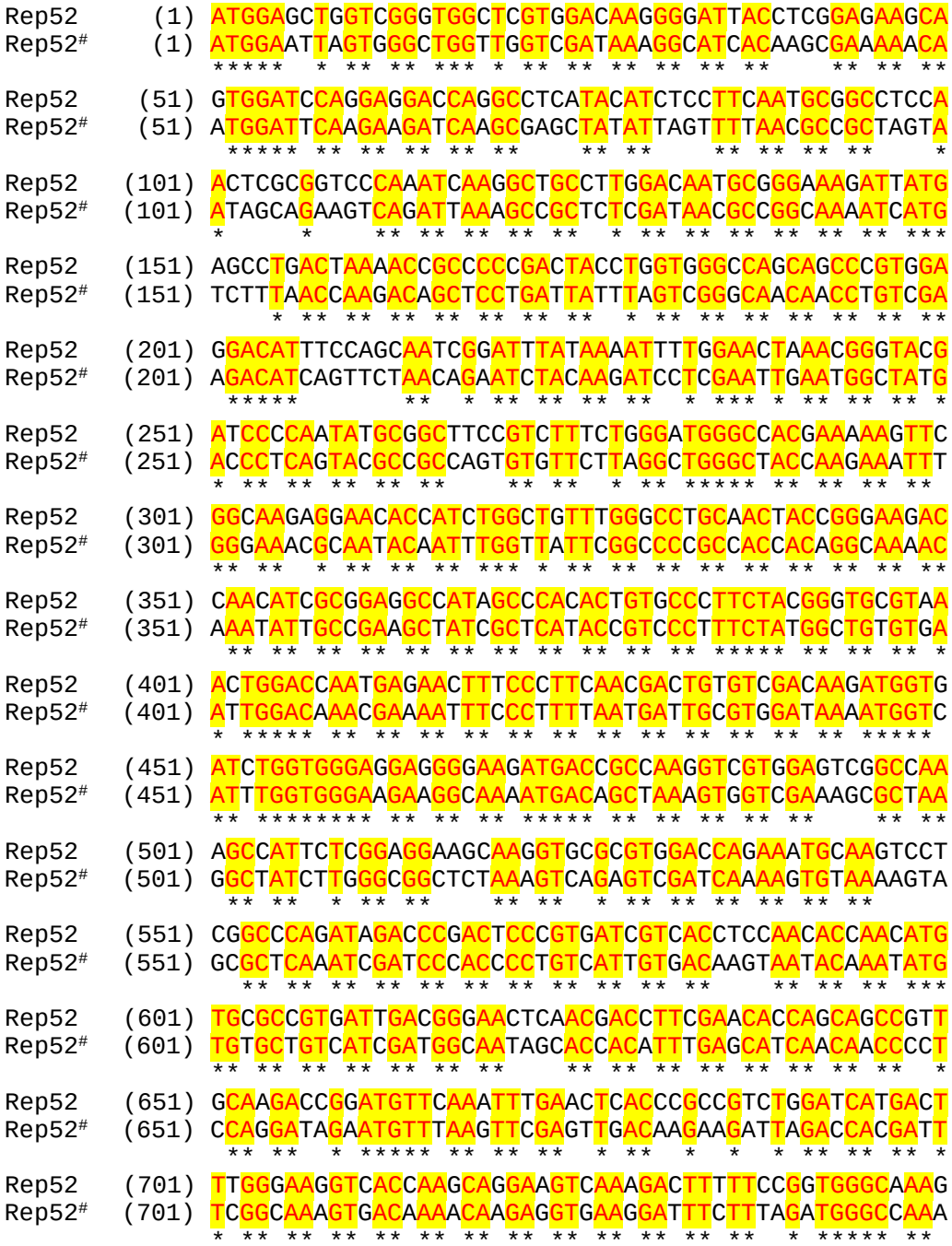
Supplemental Information

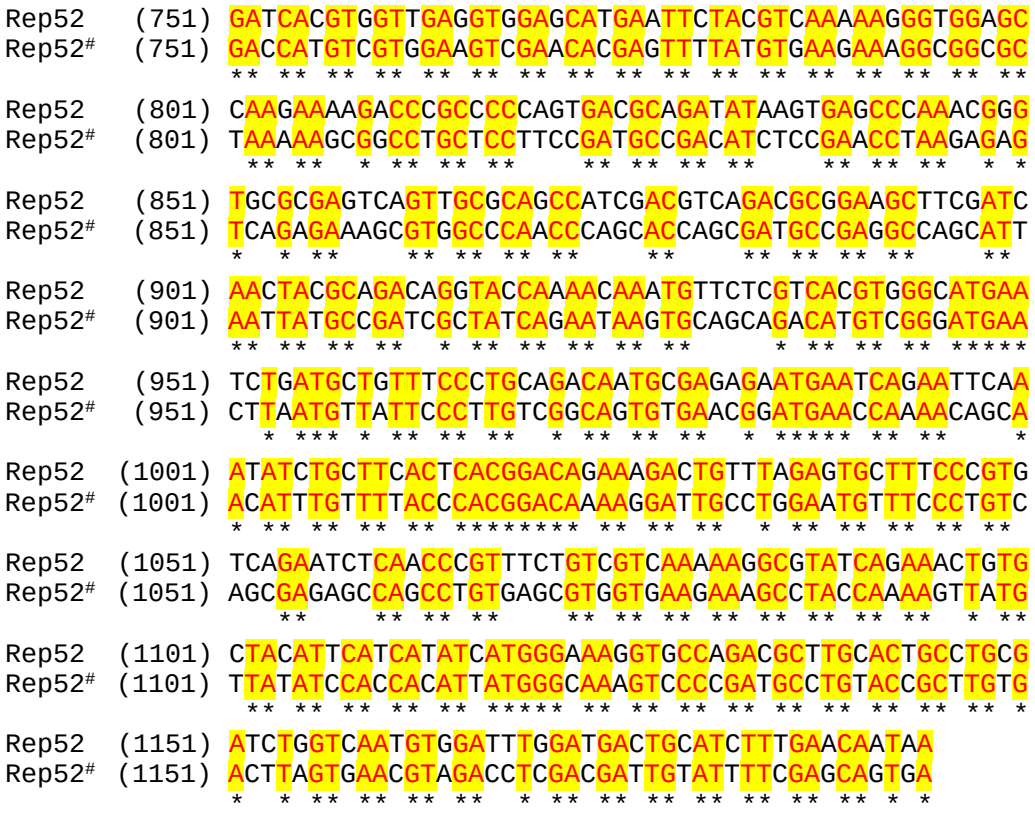
A Robust System for Production of Superabundant VP1 Recombinant AAV Vectors

Qizhao Wang, Zhongren Wu, Junping Zhang, Jenni Firrman, Hongying Wei, Zhengjing Zhuang, LinShu Liu, Linqing Miao, Yang Hu, Dong Li, Yong Diao, and Weidong Xiao

Supplementary Figure S1：Codon Optimization of Rep52 from AAV2

‘Rep52’ is original Rep52 of AAV2
‘Rep52#’ is optimized Rep52 of AAV2
***** indicates that the base remains unchanged



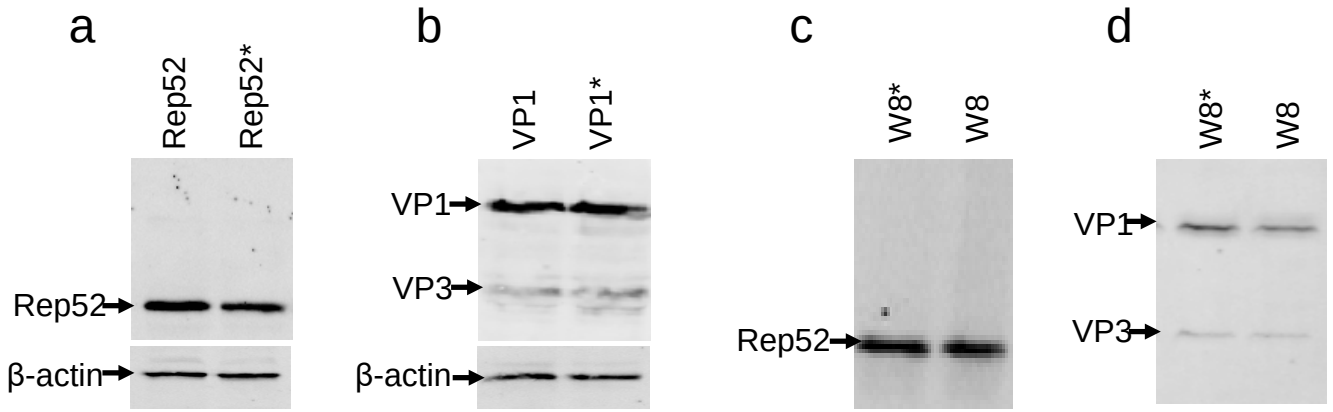

Rep52 (751) GATCACGTGGTTGAGGTGGAGCATGAATTCTACGTCAAAAAGGGTGGAGC
Rep52# (751) GACCATGTCGTGGAAGTCGAACACGAGTTTTATGTGAAGAAAGGCGGCGC
Rep52 (801) CAAGAAAAGACCCGCCCCAGTGACGCAGATATAAGTGAGCCCAAACGGG
Rep52# (801) TAAAAAGCGGCCTGCTCCTTCCGATGCCGACATCTCCGAACCTAAGAGAG
Rep52 (851) TGCGCGAGTCAGTTGCGCAGCCATCGACGTCAGACGCGGAAGCTTCGATC
Rep52# (851) TCAGAGAAAGCGTGGCCCAACCCAGCACCAGCGATGCCGAGGCCAGCATT
Rep52 (901) AACTACGCAGACAGGTACCAAAACAAATGTTCTCGTCACGTGGGCATGAA
Rep52# (901) AATTATGCCGATCGCTATCAGAATAAGTGCAGCAGACATGTCGGGATGAA
Rep52 (951) TCTGATGCTGTTTCCCTGCAGACAATGCGAGAGAATGAATCAGAATTCAA
Rep52# (951) CTTAATGTTATTCCCTTGTCGGCAGTGTGAACGGATGAACCAAAACAGCA
Rep52 (1001) ATATCTGCTTCACTCACGGACAGAAGACTGTTTAGAGTGCTTTCCCGTG
Rep52# (1001) ACATTTGTTTTACCCACGGACAAAAGGATTGCCTGGAATGTTTCCCTGTC
Rep52 (1051) TCAGAATCTCAACCCGTTTCTGTCGTCAAAAAGGCGTATCAGAAACTGTG
Rep52# (1051) AGCGAGAGCCAGCCTGTGAGCGTGGTGAAGAAAGCCTACCAAAAGTTATG
Rep52 (1101) CTACATTCATCATATCATGGGAAAGGTGCCAGACGCTTGCACTGCCTGCG
Rep52# (1101) TTATATCCACCACATTATGGGCAAAGTCCCCGATGCCTGTACCGCTTGTG
Rep52 (1151) ATCTGGTCAATGTGGATTTGGATGACTGCATCTTTGAACAATAA
Rep52# (1151) ACTTAGTGAACGTAGACCTCGACGATTGTATTTCGAGCAGTGA

Supplementary Figure2: Codon Optimization of VP1 from AAV2

'VP1' is original VP1 of AAV2
'VP1$^{\#}$' is optimized VP1 of AAV2
***** indicates that the base remains unchanged

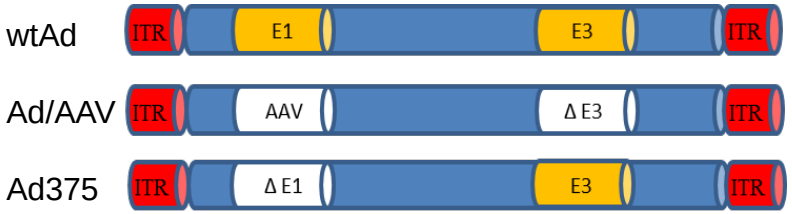

VP1# (700) GGATGGGTGATCGGGTGATTACAACATCTACAAGGACATGGGCTTTACCT
VP1 (750) ACCTACAACAACCACCTCTACAAACAAATTTCCAGCCAATCAGGAGCCTC
VP1# (750) ACATATAATAATCATTTGTATAAGCAGATCAGTTCTCAGAGCGGCGCAAG
VP1 (800) GAACGACAATCACTACTTTGGCTACAGCACCCCTTGGGGGTATTTTGACT
VP1# (800) CAATGATAACCATTATTTCGGGTATTCTACACCCTGGGGCTACTTCGATT
VP1 (850) TCAACAGATTCCACTGCCACTTTTCACCACGTGACTGGCAAGACTCATC
VP1# (850) TTAATCGGTTTCATTGTCATTTCAGCCCCAGAGATTGGCAGCGGTTGATT
VP1 (900) AACAACAACTGGGGGATTCCGACCCAAGAGACTCAACTTCAAGCTCTTTAA
VP1# (900) AATAATAATTGGGGCTTTAGGCCTAACACGGTTGAATTTTAAATTGTTCAA
VP1 (950) CATTCAAGTCAAAGAGGTCACGCAGAATGACGGTACGACGACGATTGCCA
VP1# (950) TATCCAGGTGAAGGAAGTGACCCAAACGATGGCACCACCACCATCGCTA
VP1 (1000) ATAACCTTACCAGCACGGTTCAGGTGTTTACTGACTCGGAGTACCAGCTC
VP1# (1000) ACAATTTGACATCTACCGTGCAAGTCTTCACCGATAGCGAATATCAATTG
VP1 (1050) CCGTACGTCCTCGGCTCGGCGCATCAAGGATGCCTCCCGCCGTTCCCAGC
VP1# (1050) CCCTATGTGTTGGGGAGCGCCCACCAGGGCTGTTTGCCTCCCTTTCCCGC
VP1 (1100) AGACGTCTTCATGGTGCCACAGTATGGATACCTCACCCTGAACAACGGGA
VP1# (1100) CGATGTGTTTATGGTCCCGCAATACGGCTATTTGACATTAAATAATGGCT
VP1 (1150) GTCAGGCAGTAGGACGCTCTTCATTTTACTGCCTGGAGTACTTTCCTTCT
VP1# (1150) CCCAAGCCGTGGGCAGAAGCAGCTTCTATTGTTTAGAATATTTCCCCAGC
VP1 (1200) CAGATGCTGCGTACCGGAAACAACTTTACCTTCAGCTACACTTTTGAGGA
VP1# (1200) CAAATGTTAAGAACAGGCAATAATTTCACATTCTCTTATACCTTCGAAGA
VP1 (1250) CGTTCCTTTCCACAGCAGCTACGCTCACAGCCAGAGTCTGGACCGTCTCA
VP1# (1250) TGTGCCCTTTCATTCTTCTTATGCCCATTCTCAATCCTTAGATAGATTGA
VP1 (1300) TGAATCCTCTCATCGACCAGTACCTGTATTACTTGAGCAGAACAAACACT
VP1# (1300) TGAACCCCTTGATTGATCAATATTTATACTATCTCTCTCGGACCAATACC
VP1 (1350) CCAAGTGGAACCACCACGCAGTCAAGGCTTCAGTTTTCTCAGGCCGGAGC
VP1# (1350) CCCTCCGGCACAACAACCCAAGCCGCTTGCAATTCAGCCAAGCTGGCGC
VP1 (1400) GAGTGACATTCGGGACCAGTCTAGGAACTGGCTTCCTGGACCCTGTTACC
VP1# (1400) CTCCGATATCAGAGATCAAAGCCGCAATTGGTTGCCCGGCCCTTGCTATA
VP1 (1450) GCCAGCAGCGAGT-ATCAAAGACATCTGCGGATAACAACAACAGTGAATA
VP1# (1450) GACAACAAAGGGTGAGCAAAACCAGC-GCCGACAATAATAATTCCGAGTA
VP1 (1499) CTCGTGGACTGGAGCTACCAAGTACCACCTCAATGGCAGAGACTCTCTGG
VP1# (1499) TAGCTGGACCGGCGCCACAAAATATCATTTGAACGGGCGGGATAGCTTAG
VP1 (1549) TGAATCCGGGCCCGGCCATGGCAAGCCACAAGGACGATGAAGAAAAGTTT
VP1# (1549) TCAACCCCGGGCCCGCTATGGCCTCTCATAAAGATGACGAGGAGAAATTC


VP1 (1599) TTTCCTCAGAGCGGGGTTCTCATCTTTGGGAAGCAAGGCTCAGAGAAAAC
VP1# (1599) TTCCCACAATCTGGCGTGTTGATTTTCGGCAAACAGGGGAGCGAAAAGAC
VP1 (1649) AAATGTGGACATTGAAAAGGTCATGATTACAGACGAAGAGGAAATCAGGA
VP1# (1649) CAACGTCGATATTGAGAAAGTGATGATCACCGATGAGGAAGAGATTCGCA
VP1 (1699) CAACCAATCCCGTGGCTACGGAGCAGTATGGTTCTGTATCTACCAACCTC
VP1# (1699) CCACAAACCCTGTCGCCACCGAACAATACGGCAGCGTGAGCACAAATTTG
VP1 (1749) CAGAGAGGCAACAGACAAGCAGCTACCGCAGATGTCAACACACAAGGCGT
VP1# (1749) CAACGGGGTAATCGGCAGGCCGCCACAGCCGACGTGAATACCCAGGGAGT
VP1 (1799) TCTTCCAGGCATGGTCTGGCAGGACAGAGATGTGTACCTTCAGGGGCCCA
VP1# (1799) GTTGCCCGGGATGGTGTGGCAAGATCGGGACGTCTATTTGCAAGGCCCTA
VP1 (1849) TCTGGGCAAAGATTCCACACACGGACGGACATTTTCACCCCTCTCCCCTC
VP1# (1849) TTTGGGGCCAAAATCCCTCATACCGATGGCCACTTCCATCCTAGCCCTTTG
VP1 (1899) ATGGGTGGATTCGGACTTAAACACCCTCCTCCACAGATTCTCATCAAGAA
VP1# (1899) ATGGGCGGCTTTGGCTTGAAGCATCCTCCGCCCCAAATCTTGATTAAAAA
VP1 (1949) CACCCCGGTACCTGCGAATCCTTCGACCACCTTCAGTGCGGCAAAGTTTG
VP1# (1949) TACACCCGTGCCCGCCAACCCCAGCACAACATTTTCCGCCGCCAAATTCG
VP1 (1999) CTTCCTTCATCACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCGAG
VP1# (1999) CCAGTTTCATTACCCAATATAGTACCGGCCAAGTGTCTGTCGAAATTGAA
VP1 (2049) TGGGAGCTGCAGAAGGAAAACAGCAAACGCTGGAATCCCGAAATTCAGTA
VP1# (2049) TGGGAATTACAAAAGAGAATTCTAAGAGATGGAACCCTGAGATCCAATA
VP1 (2099) CACTTCCAACTACAACAAGTCTGTTAATGTGGACTTTACTGTGGACACTA
VP1# (2099) TACCAGTAATTATAACAAAAGCGTGAACGTCGATTTCACCGTCGATACCA
VP1 (2149) ATGGCGTGTATTCAGAGCCTCGCCCCATTGGCACCAGATACCTGACTCGT
VP1# (2149) ACGGGGTCTACAGCGAACCCAGACCTATCGGGACACGGTATTTAACCAGA
VP1 (2199) AATCTGTAA
VP1# (2199) AACTTATAG

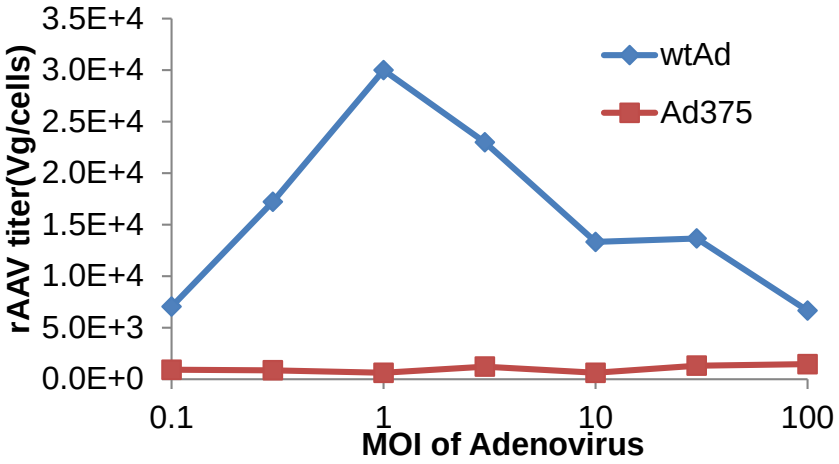


Supplementary Fig.3 Western blot analysis protein expression from codon-optimized *Rep52* and *VP1*. (a,b) Hela-S3 cells were transfected with plasmids encoding codon-optimized *Rep52*(*Rep52**) and *VP1*(*VP1**) under control of the CMV promoter. Standard *Rep52* and *VP1* were used as controls. The protein levels of Rep52 and VP1 were detected at 48 hs post transfection. (c, d) Hela-S3 cells were infected with W8 (Fig.1a) or W8* at MOI=1. The W8 vaccinia viral vector encodes regular *Rep52* and *VP1*, while the W8* encodes *Rep52** and *VP1**. All the genes were under control of the p7.5 promoter. The Rep and Cap proteins were analyzed 24 hs post-infection. In all experiment, Mouse anti-Rep and Mouse anti-VP1,VP2&VP3 were used as detecting antibodies.

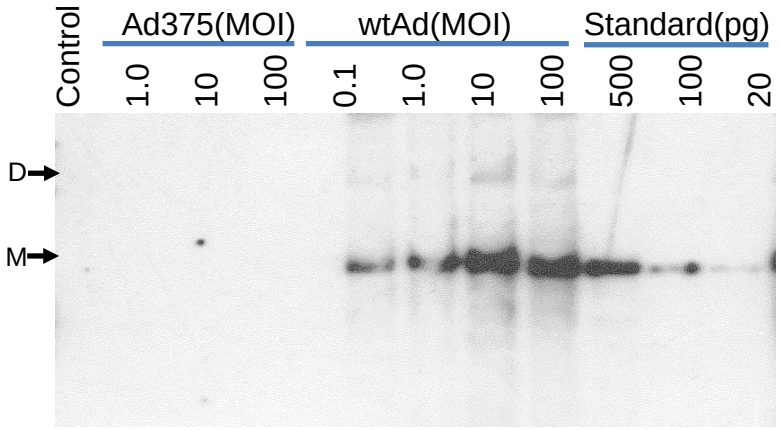
a


wtAd
ITR
E1
E3
ITR
Ad/AAV
ITR
AAV
Δ E3
ITR
Ad375
ITR
Δ E1
E3
ITR

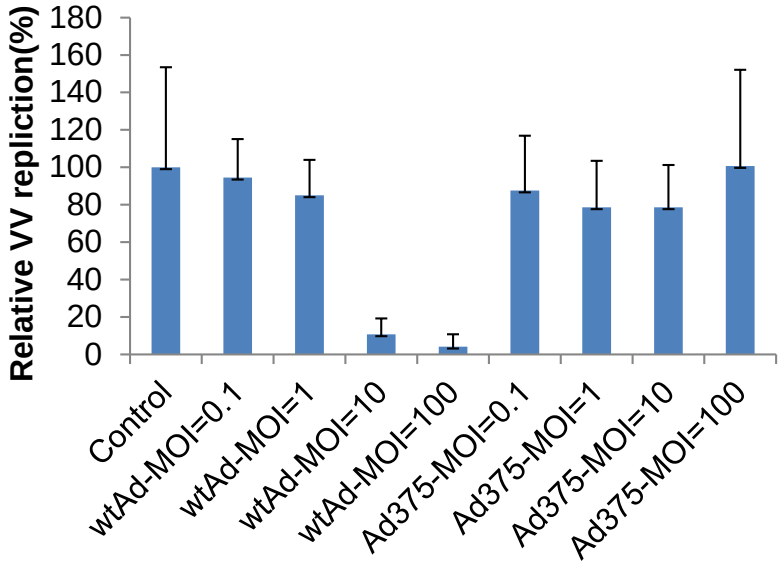
b


3.5E+4
3.0E+4
2.5E+4
2.0E+4
1.5E+4
1.0E+4
5.0E+3
0.0E+0
rAAV titer(Vg/cells)
wtAd
Ad375
0.1
1
10
100
MOI of Adenovirus

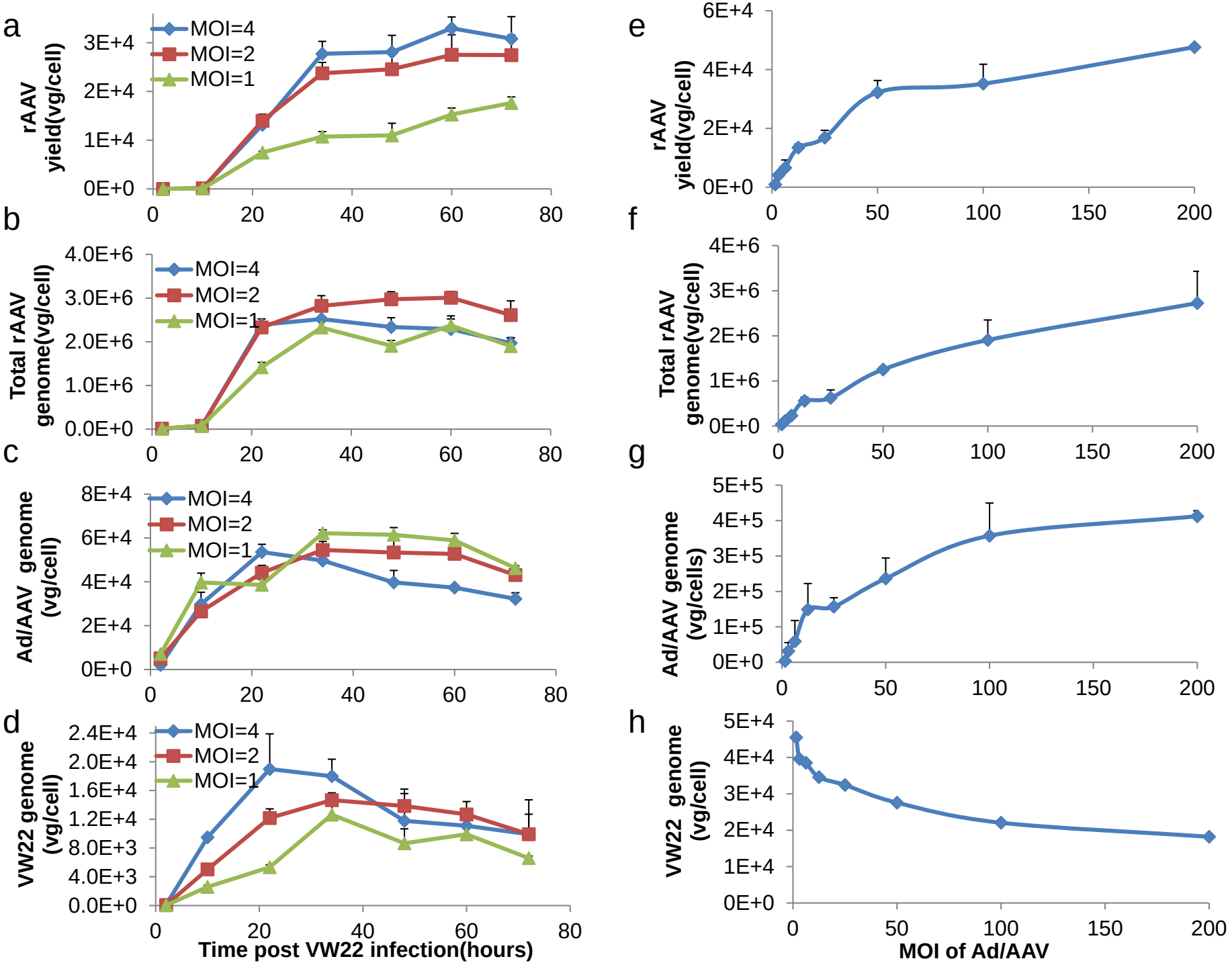
c


Control
Ad375(MOI)
1.0
10
100
wtAd(MOI)
0.1
1.0
10
100
Standard(pg)
500
100
20
D
M

d

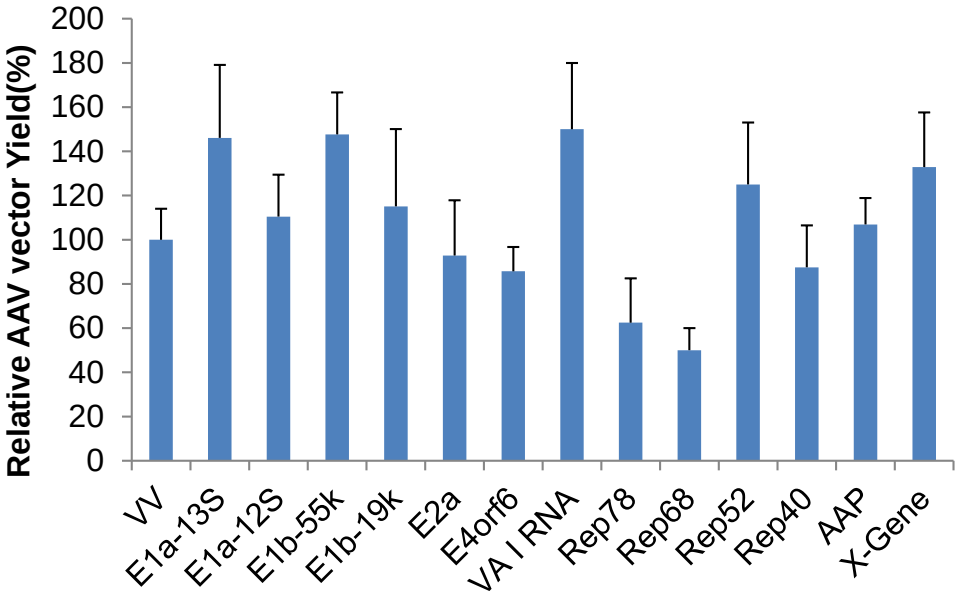

180
160
140
120
100
80
60
40
20
0
Relative VV repliction(%)
Control
wtAd-MOI=0.1
wtAd-MOI=1
wtAd-MOI=10
wtAd-MOI=100
Ad375-MOI=0.1
Ad375-MOI=1
Ad375-MOI=10
Ad375-MOI=100

Supplementary Fig.4 Effects of wtAd and Ad375 on rAAV production, rAAV genome replication, and VV genome replication in the VV-Ad system. (a) Illustration depicting the structural differences of wtAd, the Ad/AAV hybrid, and Ad375 vectors. wtAd contains both the E1 and E3 region. For the Ad/AAV hybrid vectors, the E3 region was deleted, and the E1 region was replaced with the rAAV genome. The Ad375 adenovirus contains the E3 region, but lacks E1 region. (b) The effect of wtAd and Ad375 on rAAV packaging in Hela-S3 cells. wtAd or Ad375, at different MOIs, were co-infected with Ad/AAV-CMV-EGFP 16 hours before VW22(MOI=1) infection. The rAAV vectors in Hela-S3 cells was harvested 48 hours post VW22 infection. (c) Rescue of rAAV genomes from the Ad/AAV hybrid by VW22 in the present of varied amounts of wtAd or Ad375. Southern blot was performed using a ^{32}P labelled EGFP-specific probe. The bands corresponding to the linear monomer (M) and dimer (D) of rAAV genomes are indicated. (d) Replication of VW22 was effected by wtAd, but not Ad375. The control group shows VW22 replication in VV-Ad system without the addition of any wtAd or Ad375 viruses. Bars represent the means of three independent experiments.

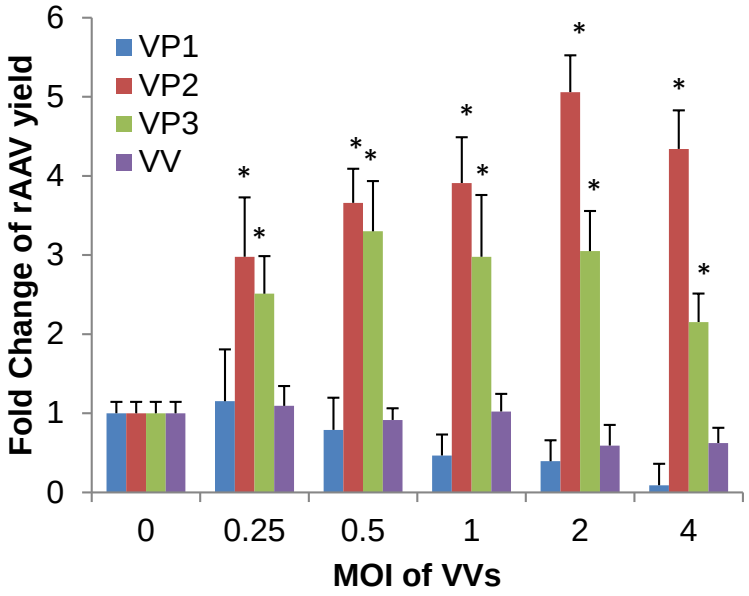

a
rAAV yield(vg/cell)
MOI=4
MOI=2
MOI=1
3E+4
2E+4
1E+4
0E+0
0
20
40
60
80
b
Total rAAV genome(vg/cell)
4.0E+6
3.0E+6
2.0E+6
1.0E+6
0.0E+0
c
Ad/AAV genome (vg/cell)
8E+4
6E+4
4E+4
2E+4
0E+0
d
VW22 genome (vg/cell)
2.4E+4
2.0E+4
1.6E+4
1.2E+4
8.0E+3
4.0E+3
0.0E+0
Time post VW22 infection(hours)
e
rAAV yield(vg/cell)
6E+4
4E+4
2E+4
0E+0
0
50
100
150
200
f
Total rAAV genome(vg/cell)
4E+6
3E+6
2E+6
1E+6
0E+0
g
Ad/AAV genome (vg/cells)
5E+5
4E+5
3E+5
2E+5
1E+5
0E+0
h
VW22 genome (vg/cell)
5E+4
4E+4
3E+4
2E+4
1E+4
0E+0
MOI of Ad/AAV

Supplementary Fig.5 Determination of the optimal multiplicity of infection (MOI) for VW22 and Ad/AAV hybrid vectors in the new VV-Ad system. The rAAV yield (a,e), total rAAV genomes(b,f), total Ad/AAV genomes(c,g), and total VW22 genomes(d,h) was determined by qPCR as mentioned in "MATERIALS AND METHODS". (a-d) Effect of VW22 MOI in the VV-Ad system on (a) rAAV yield, (b) rAAV genome accumulation, (c) Ad/AAV replication and (d) VW22 replication. Suspension QW158-7 cells (100 ml, 2e6 cells/ml) were infected with Ad/AAV-CMV-EGFP (MOI=50) and VW22 (MOI=1,2,4) was introduced 16 hs later. 1 ml of cultured sample was harvested at different points post VW22 infection. The X axis present the time points. (e-h) Effect of Ad/AAV MOI in the VV-Ad system on (e) rAAV yield, (f) rAAV genome accumulation, (g) Ad/AAV replication and (h) VW22 replication. Suspension QW158-7 cells (100 ml, 2e6 cells/ml) were infected with Ad/AAV-CMV-EGFP at varied MOIs, and VW22 (MOI=2) was introduced 16 hs later. The X axis present the MOIs of Ad/AAV-CMV-EGFP. Samples were collected 36 hours post VW22 infection. Bars represent the means of three independent experiments.

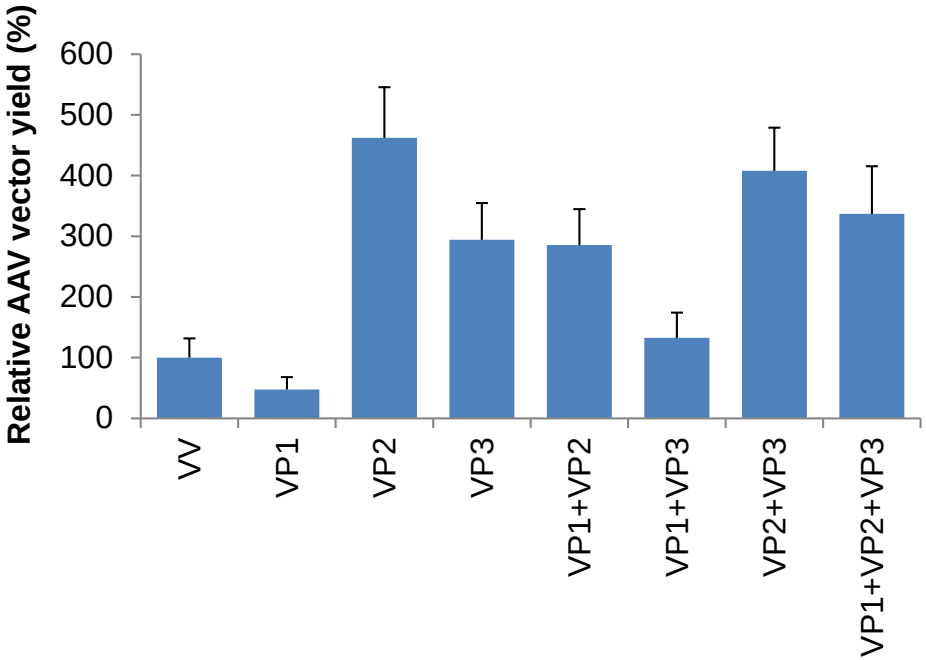
a


Relative AAV vector Yield(%)
200
180
160
140
120
100
80
60
40
20
0
VV
E1a-13S
E1a-12S
E1b-55k
E1b-19k
E2a
E4orf6
VA I RNA
Rep78
Rep68
Rep52
Rep40
AAP
X-Gene

b

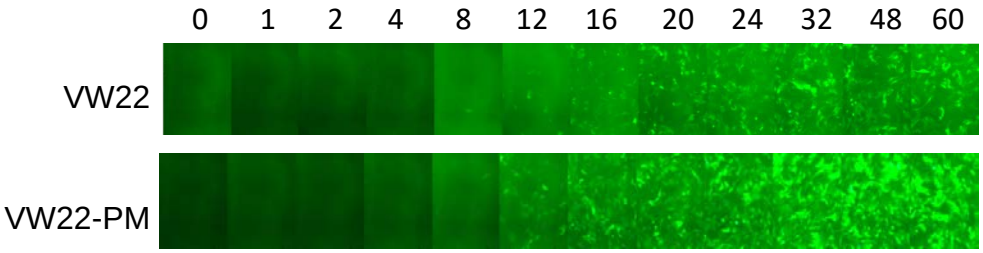

Fold Change of rAAV yield
6
5
4
3
2
1
0
VP1
VP2
VP3
VV
0
0.25
0.5
1
2
4
MOI of VVs

c

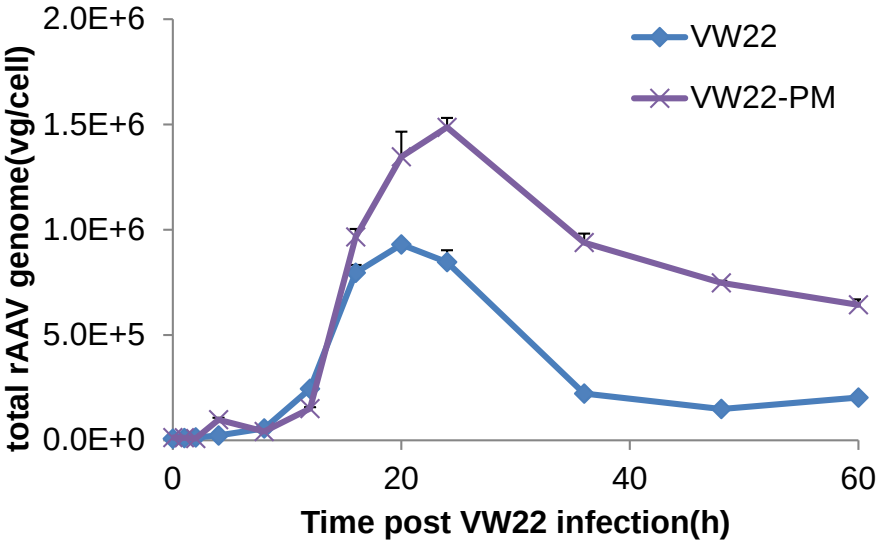

Relative AAV vector yield (%)
600
500
400
300
200
100
0
VV
VP1
VP2
VP3
VP1+VP2
VP1+VP3
VP2+VP3
VP1+VP2+VP3

Supplementary Fig.6 Screening of Ad and AAV genes that may contribute to rAAV vector production in the VV-Ad system. (a) Genes that failed to significantly increase rAAV production in the VV-Ad system. The Ad related genes included *E1a-13s*, *E1a-12s*, *E1b-55k*, *E1b-19k*, *E2a*, *VA I RNA*, and *E4orf6*. The AAV genes included *Rep78*, *Rep68*, *Rep 52*, R*ep40*, *AAP*, and *X gene*. All the genes were expressed from VV vectors under the control of p7.5 promoter and introduced to the system with MOI=1. The VV control is a vaccinia virus without any exogenous gene expression. (b) Dose-dependent effects of VP1, VP2 and VP3 on rAAV vector production. VVs carrying different genes (MOI=0~4) were co-introduced with VW22 (MOI=1) at 16 hs post Ad/AAV-CMV-EGFP (MOI=50) infection in QW158-7 cells. Y axis presents the fold changes compared with the VV control. (c) The combination effect of VP1, VP2 and VP3. Each VV was infected as MOI=1. In all the experiments, rAAV vectors were harvested at 36 h post VW22 infection and titrated by qPCR. Bars represent the means of three independent experiments.

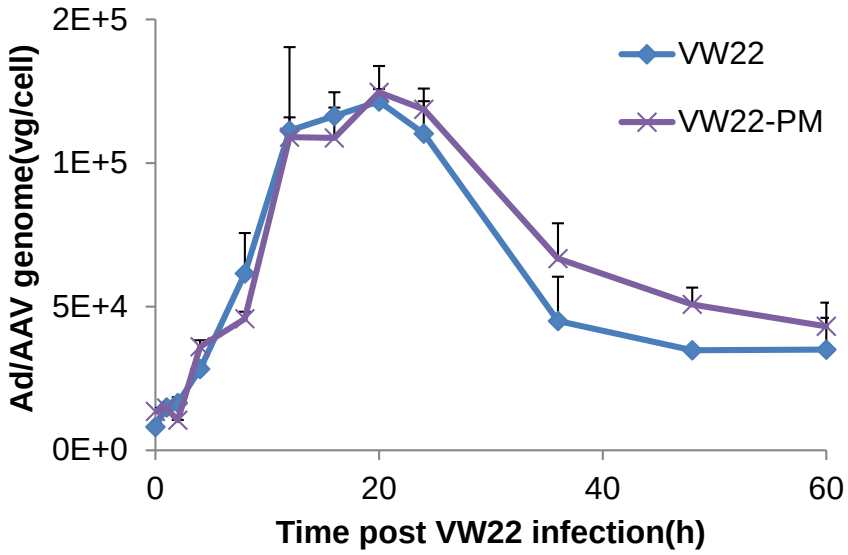
a


0 1 2 4 8 12 16 20 24 32 48 60
VW22
VW22-PM

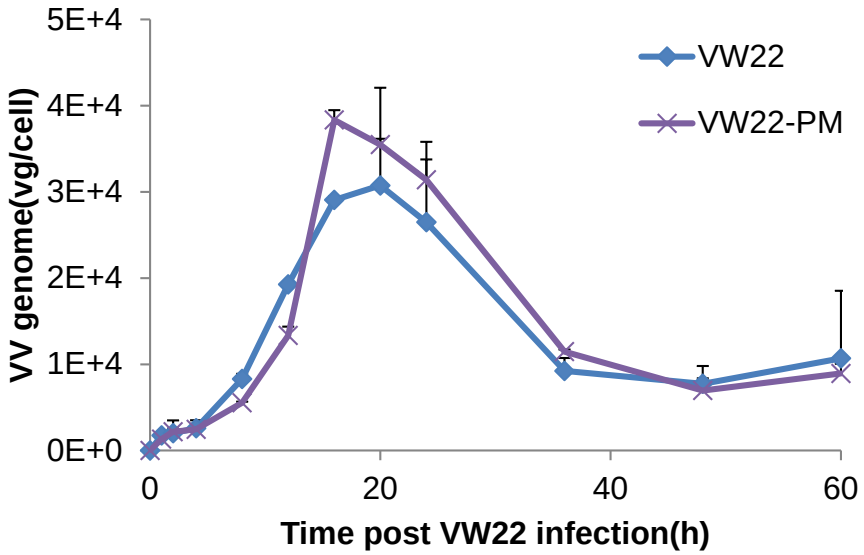
b


2.0E+6
1.5E+6
1.0E+6
5.0E+5
0.0E+0
total rAAV genome(vg/cell)
VW22
VW22-PM
0
20
40
60
Time post VW22 infection(h)

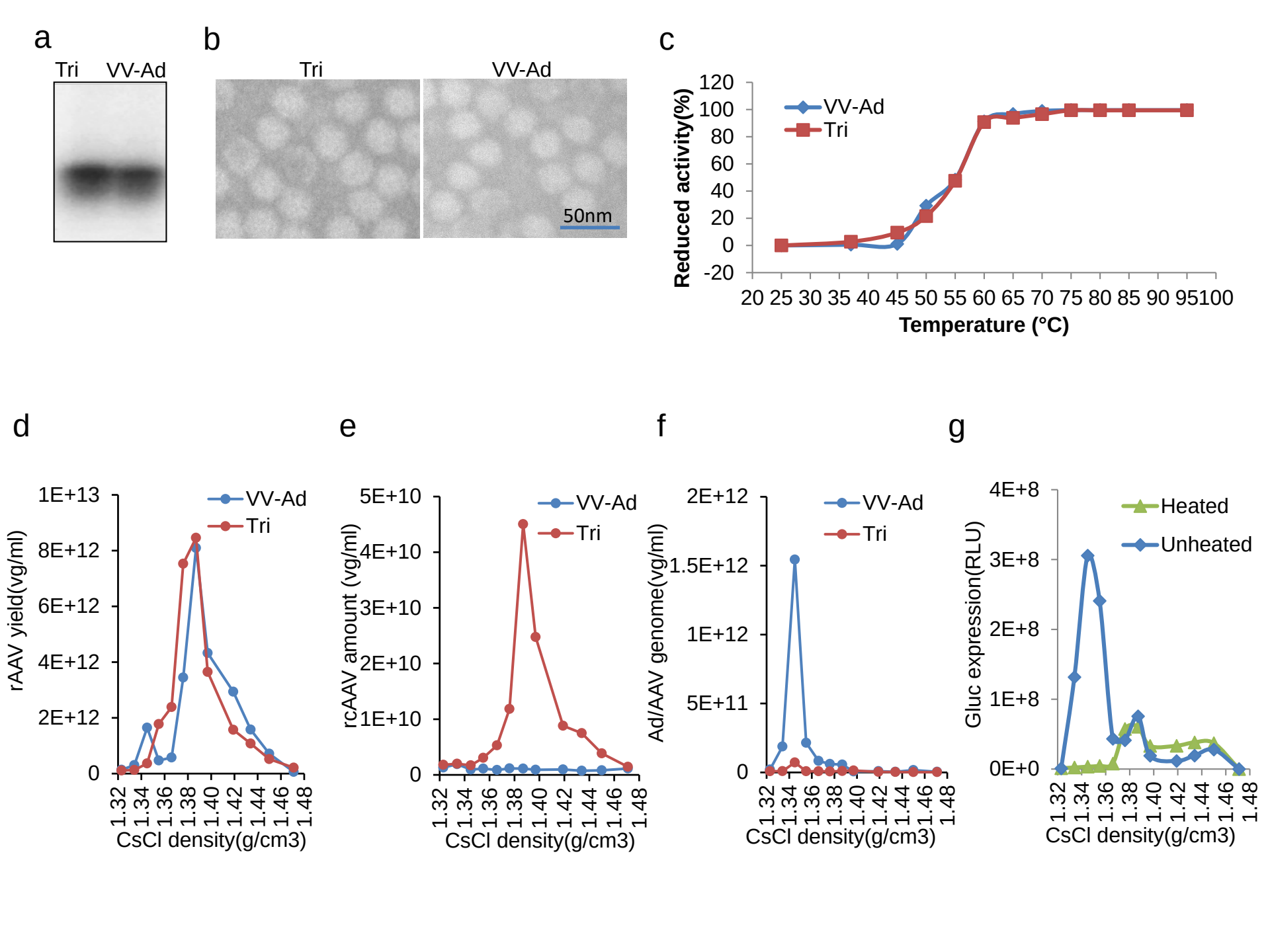
c


2E+5
1E+5
5E+4
0E+0
Ad/AAV genome(vg/cell)
VW22
VW22-PM
0
20
40
60
Time post VW22 infection(h)

d


5E+4
4E+4
3E+4
2E+4
1E+4
0E+0
VV genome(vg/cell)
VW22
VW22-PM
0
20
40
60
Time post VW22 infection(h)

Supplementary Fig.7 VW22-PM improved rAAV production in the VV-Ad system. The VW22-PM carrier a Pr4LS5E-mH5 dual promoter to drive VP2/VP3 expression, which led to earlier VP2/VP3 expression and more abundant of VP2/VP3 accumulation (Fig.3). Suspension QW158-7 cells (100 ml, 2e6 cells/ml) were infected with Ad/AAV-CMV-EGFP (MOI=50) and VW22-PM(MOI=2) or VW22 (MOI=2) was introduced 16 hs later. 1 ml of cultured sample was harvested at different points post VV infection. (a) Infectious titers of rAAV vectors produced by the VV-Ad system using either VW22 or VW22-PM. 5 µls of sample was exposed to freeze/thaw, and heat inactivation, then added into each well of a 24-well plate containing GM16095 cells. EGFP was observed 48 h post rAAV transduction. (b-d): Comparison of the effects of VW22-PM and VW22 on VV-Ad system. The titer of total rAAV vectors (b), Ad/AAVgenomes (c) and VV genomes (d) in Ad-VV system were determined by qPCR. Bars represent the means of four independent experiments.


a
Tri
VV-Ad
b
Tri
VV-Ad
50nm
c
Reduced activity(%)
120
100
80
60
40
20
0
-20
VV-Ad
Tri
20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95100
Temperature (°C)
d
rAAV yield(vg/ml)
1E+13
8E+12
6E+12
4E+12
2E+12
0
VV-Ad
Tri
1.32 1.34 1.36 1.38 1.40 1.42 1.44 1.46 1.48
CsCl density(g/cm3)
e
rcAAV amount (vg/ml)
5E+10
4E+10
3E+10
2E+10
1E+10
0
VV-Ad
Tri
1.32 1.34 1.36 1.38 1.40 1.42 1.44 1.46 1.48
CsCl density(g/cm3)
f
Ad/AAV genome(vg/ml)
2E+12
1.5E+12
1E+12
5E+11
0
VV-Ad
Tri
1.32 1.34 1.36 1.38 1.40 1.42 1.44 1.46 1.48
CsCl density(g/cm3)
g
Gluc expression(RLU)
4E+8
3E+8
2E+8
1E+8
0E+0
Heated
Unheated
1.32 1.34 1.36 1.38 1.40 1.42 1.44 1.46 1.48
CsCl density(g/cm3)

- **Supplementary Fig.8: Comparison of rAAV vectors produced by the VV-Ad system (VV-Ad) and triple plasmid transfection (Tri) methods. (**a) Southern blot analysis of the genome status of purified rAAV vectors. (b) The morphology of rAAV vectors under transmission electron microscopy (TEM). (c) The thermal stability of rAAV vectors. Thermal profile (shown as reduced activity (%) versus temperature (°C) of rAAV vectors (after 1 hour treated at different temperature) was determined by Gluc expression in GM16095 cells. (d-g) Characterization of rAAV vectors obtained from different densities of CsCl after two rounds of ultracentrifugation. (d) The distribution of rAAV vectors in CsCl gradient. rAAV genome titer in each sample was determined by qPCR. (e) rcAAV contamination in different CsCl gradient of rAAV vectors. rcAAV genomes were determined by qPCR targeting *Rep78*. (f) Determination of Ad/AAV hybrid contamination in different CsCl gradient of rAAV vectors by qPCR. (g) Inactivation of Ad/AAV hybrid vectors. “Heated” present the Gluc expression from fractions after heating at 56 ℃ for 1 hour. In this group, the Gluc activity is only expressed from rAAV vectors. The “Unheated” presents the Gluc expression of fractions without any treatment. In this group, Gluc activity could be either from rAAV vectors or from dsAd/AAV-CB-Gluc Ad/AAV hybrid.

Supplementary Table 1. Promoter sequences used in the paper.

LEO160	TTTTATTTTTTTTTTTTGGAATATAAATATCCGGTAAAATTGAAAAAATATACACTAATTAGCGTCTC GTTTCAGACGCTAGCTCGAGGTTGGGAGCTCTCCGGATCCAAGCTTATCGATTTCGAACCCGGGG TACCGAATTCCTCGAGGTTGGGAGCTCTCCGGATCCAAGCTTATCGATTTCGAACCCGGGGTACC GAATTCCTCGAG
PR4LS5E	GTTTTGAATAAAATTTTTTTATAATAAATAAATTTTTAATATATAAATATTCTGCATAAATAAAAAT ATTTTTAGCTTCTAAATATTGATCAATAGTGAAGTTATTGTCAATAAATAGTTTAAACAAAAATTGA AATTTTATTTTTTTTTTTTGGAATATAAATAAAAAATTGAAAAACTATTCTAATTTATTGCACGGTCC GGTAAAAATTGAAAAACTATTCTAATTTATTGCACGGTCCGGTAAAAATTGAAAAACTATTCTAAT TTATTGCACGGTCCGGTAAAAATTGAAAAACTATTCTAATTTATTGCACGGTCGGTAAAAATTGAA AAACTATTCTAATTTATTGCACGG
PHYB	ACGCGTGTTTAAACGTTTTGAAAATTTTTTTATAATAAATATCCGGTAAAAATTGAAAAACTATTCT AATTTATTGCACGGTCCGGAAAAATTGAAAAACTATTCTAATTTATTGCACGGTCCGGTAAAAATT GAAAAACTATTCTAATTTATTGCACGGTCCGGTCCGGTAAAAATTGAAAAACTATTCTAATTTATT GCACGGTCCGGTCCGGTAAAAATTGAAAAACTATTCTAATTTATTGCACGGTCCGGTCCGGA
PRMVA1 3.5-LONG	TAAAAATAGAAACTATAATCATATAATAGTGTAGGTTGGTAGTATTGCTCTTGTGACTAGAGACTT TAGTTAAGGTACTGTAAAAATAGAAACTATAATCATATAATAGTGTAGGTTGGTAGTA
PRS5E	AAAAATTGAAATTTTATTTTTTTTTTTTTGGAATATAAATAAAAAATTGAAAAACTATTCTAATTTATT GCACGGTCCGGTAAAAATTGAAAAACTATTCTAATTTATTGCACGGTCCGGTAAAAATTGAAAAA CTATTCTAATTTATTGCACGGTCCGGTAAAAATTGAAAAACTATTCTAATTTATTGCACGGTCCGGT AAAAATTGAAAAACTATTCTAATTTATTGCACGG
MH5	CTCGAGAAAAATTGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTAAATTGAAAGCGAGAAAT AATCATAAATAA
ATI	GTTTTGAATAAAATTTTTTTATAATAAAT
PS	AAAATTGAAATTTTATTTTTTTTTTTTTGGAATATAAATAGCTAGCTCGAG
P7.5	AAAAATTGAAATTTTATTTTTTTTTTTTGGAATATAAAT