Supplementary Material **Applied Microbiology and Biotechnology**

Enhanced ER Protein Processing Gene Expression Increases rAAV Yield and Full Capsid Ratio in HEK293 Cells

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Supplementary Table S1. Plasmid information.

Plasmid name	**Description**
pU6-(BbsI)_CBh-Cas9-T2A-mCherry	Backbone of sgRNA-Cas9 vectors (Addgene plasmid #64324, Watertown, United States)
AAVS1-sgRNA-Cas9	sgRNA targeting *AAVS1* locus and Cas9 expression vector
AAVS1-eGFP donor	Donor plasmid targeting *AAVS1* locus (GOI: *eGFP*)
AAVS1-BCL2 donor	Donor plasmid targeting *AAVS1* locus (GOI: *BCL2*)
AAVS1-XBP1 donor	Donor plasmid targeting *AAVS1* locus (GOI: *XBP1*)

AAVS1-HSPA6 donor	Donor plasmid targeting *AAVS1* locus (GOI: *HSPA6*)
AAVS1-GADD34 donor	Donor plasmid targeting *AAVS1* locus (GOI: *GADD34*)
pAdDeltaF6	Plasmid contains helper genes E2, E4 and VARNA for rAAV production (Addgene plasmid #112867, Watertown, United States)
pAAV2/2	Plasmid contains Rep and Cap genes for AAV2 production (Addgene plasmid #104963, Watertown, United States)
pAAV2/8	Plasmid contains Rep and Cap genes for AAV8 production (Addgene plasmid #112864, Watertown, United States)
AAV-CMV-*GFP*	Plasmid contains CMV-*GFP* flanked by ITRs for rAAV production (Addgene plasmid #67634, Watertown, United States)
Plasmid name (PCR template)	**Elements**
AAVS1-eGFP donor	*AAVS1* backbone, including: homology arms, *puromycin* resistance gene, CMV promoter, SV40 poly(A)
pMSCV*puro-BCl2*	*BCL2* gene, (Addgene plasmid #191964, Watertown, United States)
pcDNA5/FRT/TO *GFP HSPA6*	*HSPA6* gene, (Addgene plasmid #19486, Watertown, United States)
pcDNA3.1+/C-(K)DYK *XBP1*(NM_005080) ORF Clone	*XBP1* gene, (Genscript, OHU26371D, Piscataway, United States)
pcDNA3.1+/C-(K)DYK *PPP1R15A*(NM_014330) ORF Clone	*GADD34* gene, (Genscript, OHU17374D, Piscataway, United States)

Supplementary Table S2. Primer information.

Primer name	**Description**	**Sequence (5'-3')**
AAVS1 sgRNA fwd	Sense oligo for *AAVS1*-sgRNA	CACCGACCCCACAGTGGGGCCACTA

*AAVS1*_sgRNA_rev	Antisense oligo for *AAVS1*-sgRNA	AAACTAGTGGCCCCACTGTGGGGTC
AAVS1 donor backbone fwd	Sense oligo for *AAVS1* donor backbone	GGGATCCACCGGATCTAGATAACT
AAVS1 donor backbone rev	Antisense oligo for *AAVS1* donor backbone	GGCGGTTTCACTAAACCAGCTCTGCTT
*XBP1*_fwd	Sense oligo for *XBP1*	ACCGCCATGGTGGTGGTGGCAGCCG
*XBP1*_rev	Antisense oligo for *XBP1*	TTAGTTCATTAATGGCTTCCAGCT
*GADD34*_fwd	Sense oligo for *GADD34*	ACCGCCATGGCCCCAGGCCAAGCAC
*GADD34*_rev	Antisense oligo for *GADD34*	TCAGCCACGCCTCCCACTGA
*BCL2*_fwd	Sense oligo for *BCL2*	GCCACCATGGCGCACGCTGGGAGA
*BCL2*_rev	Antisense oligo for *BCL2*	TCACTTGTGGCCCAGATAGGC
*HSPA6*_fwd	Sense oligo for *HSPA6*	GCCACCATGCAGGCCCCACGGGAG
*HSPA6*_rev	Antisense oligo for *HSPA6*	TCAATCAACCTCCTCAATGA
*BCL2*_3' junction_fwd	*AAVS1* amplicon for 3' junction PCR	GTTTGATTTCTCCTGGCTGTCTCT
*HSPA6*_3' junction_fwd	*AAVS1* amplicon for 3' junction PCR	AATGCAAGACAAGTGTCGGGAAG
*GADD34*_3' junction_fwd	*AAVS1* amplicon for 3' junction PCR	ACTGTCCATTTCCTGGCTGTCT
*XBP1*_3' junction_fwd	*AAVS1* amplicon for 3' junction PCR	TGAGAACCAGGAGTTAAGACAGCG
*AAVS1*_3'junction_rev	*AAVS1* amplicon for 3' junction PCR	CCAGCCTCACCAAGTGGTTCATAA

*AAVS1*_5'junction_fwd	*AAVS1* amplicon for 5' junction PCR	TACTCTCTTCGATTGGAGTCGCTT
*puro*_5' junction_rev	*AAVS1* amplicon for 5' junction PCR	CGCGTGAGGAAGAGTTCTTGCA
XBP1 qPCR fwd	*XBP1* amplicon for qRT- PCR	GCTGGAACAGCAAGTGGTAG
XBP1 qPCR rev	*XBP1* amplicon for qRT- PCR	CCACTGGCCTCACTTCATT
BCL2 qPCR fwd	*BCL2* amplicon for qRT- PCR	AGGATTGTGGCCTTCTTTGA
BCL2 qPCR rev	*BCL2* amplicon for qRT- PCR	ACAGTTCCACAAAGGCATCC
HSPA6 fwd	*HSPA6* amplicon for qRT- PCR	CACCAAGCAGACCCAGACTT
HSPA6 rev	*HSPA6* amplicon for qRT- PCR	CACTGAGTTCAAAACGCCCC
GADD34 fwd	*GADD34* amplicon for qRT- PCR	CTGGCTGGTGGAAGCAGTAA
GADD34 rev	*GADD34* amplicon for qRT- PCR	TATGGGGGATTGCCAGAGGA
GAPDH fwd	*GAPDH* amplicon for qRT- PCR	CCCACCACACTGAATCTCCC
GAPDH rev	*GAPDH* amplicon for qRT- PCR	TACATGACAAGGTGCGGCTC

Supplementary Table S3. Transfection parameters for low-producing condition

6 well plate transfection conditions (low producing condition)	
Media working volume	3 mL
Plating density (total cells)	5.00E+05
Total DNA amount	2 µg
Plasmid ratio (pHelper:pAAV2/2:pGFP)	1:1:1
PEI:DNA	1:1
Cocktail volume	300 µL

Incubation time	15 min
Cocktail media	OptiMEM

Supplementary Table S4. Transfection parameters for high-producing condition

6 well plate transfection conditions (high producing condition)	
Media working volume	2 mL
Plating density (total cells)	5.00E+05
Total DNA amount	4 µg
Plasmid ratio (pHelper:pAAV2/2:pGFP)	2:1.5:1
PEI:DNA	1:1
Cocktail volume	200 µL
Incubation time	15 min
Cocktail media	OptiMEM

Supplementary Table S5. Viable cell density (VCD) and cell viability for AAV2 high-producing condition in 293T cells

	Sample	**VCD**	**Viability**
BCL2	1	1.44E+06	87.30%
	2	1.64E+06	90.10%
	3	1.64E+06	84.60%
XBP1	1	1.53E+06	88.00%
	2	1.72E+06	90.20%
	3	9.60E+05	88.70%
HSPA6	1	1.48E+06	90.40%
	2	1.38E+06	89.80%
	3	9.57E+05	89.70%
GADD34	1	1.98E+06	89.90%
	2	1.76E+06	86.50%
	3	1.34E+06	89.20%

Parental	1	1.80E+06	87.30%
	2	2.18E+06	88.60%
	3	* missing	N/A

Note: N/A: * Samples for VCD and viability evaluation were missing, so no results can be shown here.

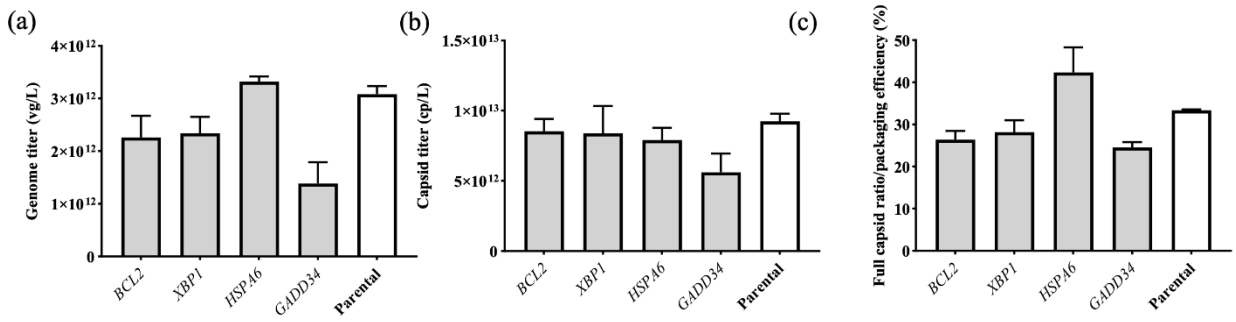
Supplementary Table S6. Viable cell density (VCD) and cell viability for AAV8 high-producing condition in 293T cells

	Sample	**VCD**	**Viability**
BCL2	1	1.74E+06	91.40%
	2	1.52E+06	86.80%
	3	1.55E+06	93.00%
XBP1	1	9.77E+05	97.70%
	2	9.21E+05	92.10%
	3	8.61E+05	86.10%
HSPA6	1	1.04E+06	96.20%
	2	1.18E+06	92.70%
	3	9.29E+05	92.00%
GADD34	1	1.23E+06	90.90%
	2	1.23E+06	90.20%
	3	1.01E+06	91.50%
Parental	1	1.04E+06	67.00%
	2	9.00E+05	70.80%
	3	1.88E+06	92.80%

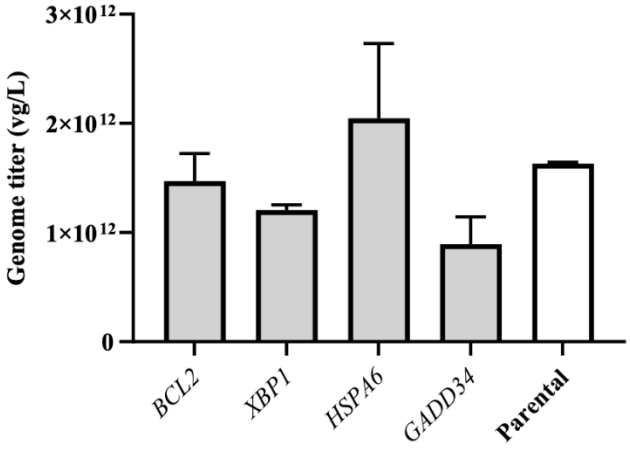
Supplementary Table S7. Viable cell density (VCD) and cell viability for AAV2 high-producing condition in HEK293 cells

	Sample	**VCD**	**Viability**
BCL2	1	9.53E+05	85.90%

	2	1.12E+06	95.50%
	3	8.14E+05	92.10%
XBP1	1	1.16E+06	86.90%
	2	1.37E+06	88.10%
	3	1.51E+06	87.50%
HSPA6	1	1.79E+06	92.50%
	2	1.34E+06	93.10%
	3	1.94E+06	97.70%
GADD34	1	1.28E+06	88.70%
	2	1.39E+06	94.40%
	3	1.42E+06	93.80%
Parental	1	1.63E+06	87.50%
	2	1.07E+06	88.90%
	3	2.11E+06	95.00%



Supplementary Figure S1. Triple plasmid transient transfection for AAV2 production in HEK293T cells under low producing condition for both overexpressed stable pools and parental cells. Genome titers were evaluated for each condition. The error bars represent the biological duplicates. (a) Genome titer in vg/L. (b) Capsid titer in cp/L. (c) Packaging efficiency.



Supplementary Figure S2. Triple plasmid transient transfection for AAV2 production in HEK293 cells under low producing condition for both overexpressed stable pools and parental cells. Genome titers were evaluated for each condition at harvest. The error bars represent biological duplicates.