Appendices

PCR programs

Table A1: InFusion PCR program

Step	Temperature	Time
1	98ºC	10 sec
2	60ºC	10 sec
3	72ºC	30 sec
4	Go to Step 1, 34 times	
5	72ºC	10 min
6	4ºC	∞

Table A2: Short-range (SR; amplicon size< 1kb) and Mid-range (MIDR; for amplicon size= 1-5kb) PCR program

Step	Temperature	Time
1	93ºC	3 min
2	92ºC	15 sec
3	65ºC	30 sec
	-1ºC/ cycle	
4	65ºC	2 min (SR) 4min (MIDR)
5	Go to Step 2, 8 times	
6	92ºC	15 sec
7	55ºC	30 sec
8	65ºC	2 min (SR) 4min (MIDR)
	+20 sec/ cycle	
9	Go to Step 6, 30 times	
10	65ºC	9 min
11	10ºC	∞

Table A3: Recombineering PCR program

Step	Temperature	Time
1	95°C	2 min
2	95°C	30 sec
3	60°C	30 sec
4	72°C	2 min 30 sec
5	Go to Step 2, 4 times	
6	95°C	30 sec
7	72°C	2 min 30 sec
	+5 sec/ cycle	
8	Go to Step 6, 27 times	
9	72°C	7 min
10	4°C	∞

Table A4: Sequencing PCR program

Step	Temperature	Time
1	96°C	45 sec
2	92°C	10 sec
3	52°C	10 sec
4	60°C	2 min
5	Go to Step 2, 59 times	
6	10°C	∞

Primers and oligo sequences

Table A5: AAAS2 KO oligos and primers

Oligo	Sequence
CRISPR gRNA oligos (U6_gRNA (AU flip) cloning append is underlined)	
CRISPR 1	5'-ACCGGATTTGGTGAGGCATGGCAAA
CRISPR 2	5'-ACCGGCACAAAAGTGAGGAGTGTGA

CRISPR 3	5’- ACCGGATCCCCTAAAGACCCCTGGA
CRISPR 4	5’- ACCGGTGAAGGCAGTTCTTGTGCCA
InFusion PCR primers (pUC19_RV InFusion cloning append is underlined)	
5F	5'-GCCAGTGAATTCGATGGGAGGATTAATTTGGTACTTCCCT
3R	5'-TACGCCAAGCTTGATTGTAGAGAAAAGAACATGGTTTCGC
Recombineering primers (50-bp append from AAAS InFusion clone is underlined)	
U5	5’-TTTGTGGAAATGGGAAACAGACCTCTGGAATCTCTTATACTTAGCCCAGCAAGGCGCATAACGATACCAC
D3	5’ TTGAAGAACACCCCAAGGTAAAGGGGTGTTGACTCATTTTCCAGGATCTGCCGCCTACTGCGACTATAGA
PCR primers	
F1	5’- CATGGTGTTCCTGGGAAGGACAAGG
R1	5’- GAAGTGGTAATGCCATGAGGTGGGG
F2	5’- GTCTGGCGGAGTGGAAACAGCTTGG
R2	5’- GAAGTTGCAGTGAGCTGAGCACGCC
F3	5’- TCCAGCCAGCTGCCTGTGACATAGC
R3	5’- TCAGTGCCACTGCACTGGCACAATC
Sequencing primers	
Seq_F	5’- GAGATGGCACGGAATTAAAG
Seq_R	5’- TCTGCAGACTGTGACCCAGG

Table A6: AAAS1 point mutation oligos and primers

Oligo	**Sequence**
ssODN (101 bases)	
C>A mutation oligo	5’–TACTGCCCGTCACCAGCTCGTTATTGTGCTCATATAGGGTGACTT**T**ACCCCGAGGCGGTGGAGGAGGGAACAACCCCAGAGAGCACATCTTGCCGGTTCG – 3’

C>A + PAM mutation oligo	5'– TACTGCCCGTCACCAGCTCGTTATTGTGCTCATATAGGGTG ACTT**T**ACCCCGAG**A**CGGTGGAGGAGGGAACAACCCCAGAG AGCACATCTTGCCGGTTCG – 3'
crRNA (20 base guide sequence)	5' – TATAGGGTGACTTGACCCCG… – 3'
PCR primers	
F1	5'- GCAGCACTGTTCCTCCTCTCTGAGG
R1	5'- CTTGTCCTTCCCAGGAACACCATGG
F2	5'- AATTTGGTACTTCCCTGAATGTGGC
R2	5'- TAGTTCTCCCGCATCCTCACACTCC
Sequencing primers	
Seq_F	5'- GCGGTCTGTGCCGTTCCGGC
Seq_R	5'- TCCCTCCTCGCCCTGGCCAC

Table A7: HTT oligos and primers

Oligo	**Sequence (20 base guide sequence)**
CRISPR gRNA oligos (U6_gRNA (AU flip) cloning append is underlined)	
CRISPR 1	5'-ACCGGCCTCCGGGGACTGCCGTGC
CRISPR 2	5'-ACCGGTCGCCGGCCCGCAGGCTGC
crRNAs	
crRNA 1	5'-GCCTCCGGGGACTGCCGTGC…
crRNA 2	5'-GTCGCCGGCCCGCAGGCTGC…
crRNA 3	5'-GAAGGACTTGAGGGACTCGA…
crRNA 4	5'-CTTTTCCAGGGTCGCCATGG…
InFusion PCR primers (pUC19_RV InFusion cloning append is underlined)	
F1	5'-GCCAGTGAATTCGATACGCCCCTACCTCACCAC
R1	5'-TACGCCAAGCTTGATTCAGGCTGTTTTAAGTGCCAC
F2	5'-GCCAGTGAATTCGATCCATTACAGTCTCACCACGC
R2	5'-TACGCCAAGCTTGATACTCATTCAAACGCCTGCAG
PCR primers	

F3	5’- TCGCCACGCCTCCCTTACCATGCAG
R3	5’- CCACAACTCATTCAAACGCCTGCAG
F4	5’- TCACACTTGGGGTCCTCAGGTCGTG
R4	5’- AACCTCCCCATCAGCAACGTGTTGG
F5	5’- TTTTACCTGCGGCCCAGAGC
R5	5’- CAAACTCACGGTCGGTGCAG
Sequencing primers	
Seq_F	5’-CAGAGCCCCATTCATTGCC
Seq_R	5’-CCCAAACTCACGGTCGGT

Table A8: Plasmid specific PCR and sequencing primers

Puro-cassette PCR primers	
Forward (EF)	5’-CATGTCTGGATCCGGGGGTACCGCGTCGAG
Reverse (ER)	5’- GCGATCTCTGGGTTCTACGTTAGTG
pUC19_RV sequencing primers	
pUC19_for	5’- AACTGTTGGGAAGGGCGATC
pUC19_rev	5’- GTTAGCTCACTCATTAGGCAC
U6_gRNA (AU flip) sequencing primers	
U6_for	5’- TCAGGAGAGCGTTCACCGAC
U6_rev	5’- CTGTTTATGTAAGCAGACAG

Plasmid maps and sequences

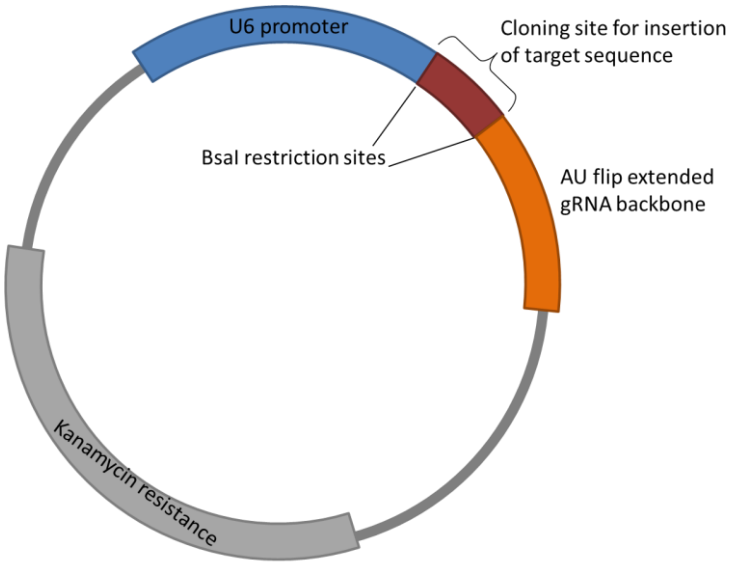

Figure A9: U6_gRNA (AU flip) expression plasmid containing extended guide RNA backbone

Sequence of U6_gRNA (AU flip) expression plasmid

CTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCTAGCCAGGAAGAGT
TTGTAGAAACGCAAAAAGGCCATCCGTCAGGATGGCCTTCTGCTTAGTTTGATGCCTG
GCAGTTTATGGCGGGCGTCCTGCCCGCCACCCTCCGGGCCGTTGCTTCACAACGTTCAA
ATCCGCTCCCGGCGGATTTGTCCTACTCAGGAGAGCGTTCACCGACAAACAACAGATA
AAACGAAAGGCCCAGTCTTCCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCAGTTCC
CTACTCTCGCGTTAACGctagcatggatctcgggccATTAACCCTCACTAAAGGGAAAGGTCGGG
CAGGAAGAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGT
TAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACG
TGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAAT
GGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTT
GTGGAAAGGACGAAACACCGggagaccgaattcgagagggtctcaGTTTAAGAGCTATGCTGGAAA
CAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAG
TCGGTGCTTTTTTTTTCATATGTGTCACCTAAATtgcagctcTGGCCCGTGTCTCAAAATCTC
TGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTT
ACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGC
CGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAA
TGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAG
TTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCA
GACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACT
CCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGAAAAACAGCATTCCAGGTAT
TAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCG
CCGGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCT
CGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGAC
GAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCAT
TCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACG
AGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCA
GGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGC
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TCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT
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TGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCA
GATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTG
TAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGC
GATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGC
GGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACA
CCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGA
GAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGG
GAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTG
ACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCC
AGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTT

Legend: Red – AU flip extended gRNA backbone
Yellow highlighted: cloning site for insertion of target sequence

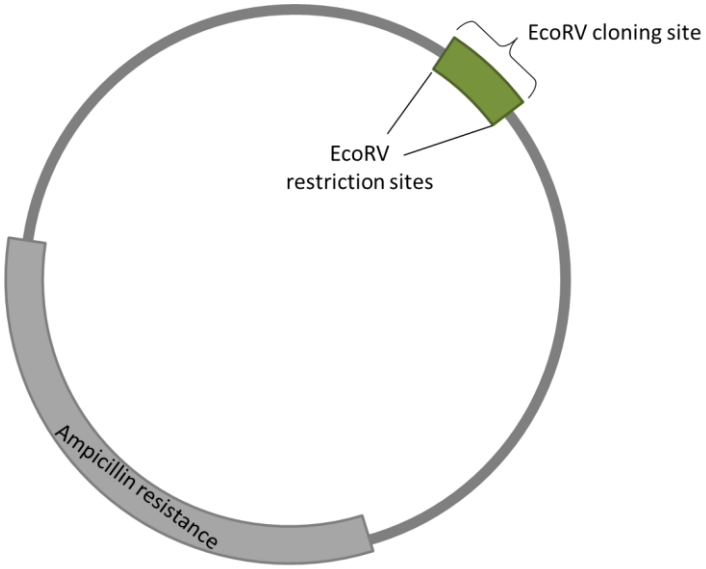

Figure A10: pUC19_RV plasmid containing EcoRV linearization site

Sequence of pUC19_RV plasmid

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGT
CACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGC
GGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTG
AGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGC
ATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGG
CCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTG
GGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCGAT
ATCGGCGCGCCGATATCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAA
TTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCC
TGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTT
CCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAG
AGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGG
TCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCAC
AGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCA
GGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGA
GCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAG
ATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGC
TTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCA
CGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGA

ACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACC
CGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG
CGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACAC
TAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGA
GTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTG
CAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCT
ACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGAT
TATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATC
TAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCAC
CTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGA
TAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGA
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CGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGA
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GCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGA
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CTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCA
CTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTA
CTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCG
TCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAA
AACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGAT
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GGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGG
AAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTAT
TGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTC
CGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGAC
ATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

Legend: Yellow highlighted: EcoRV linearization site for cloning

A11: Sequence of pL1L2_ EF1α-puro-polyA plasmid

CTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCTAGCATGGATCTCG
GGGACGTCTAACTACTAAGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAA
GGCTCAGTCGGAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTCTCC
TGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGTGAAGCAACGGCCCGGAG
GGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAACTAAGCAGAAGGCCA
TCCTGACGGATGGCCTTTTTGCGTTTCTACAAACTCTTCCTGTTAGTTAGTTACTTAAGC
TCGGGCCCCAAATAATGATTTTATTTTGACTGATAGTGACCTGTTCGTTGCAACAAATT
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TTGGGGAATCCCAGGGACCGTCGTTAAACTCCCACTAACGTAGAACCCAGAGATCGCT
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GTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAA
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CGTGCTTGAGTTGAGGCCTGGCTTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGC
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CTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCAC
ACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCA

CATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGT
CTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCC
TGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTT
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GGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTG
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TACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGT
GGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCC
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CTTCCATTTCAGGTGTCGTGATCTAGATGTCGAGCGGCCGCGGCGGCGCCCTATAAAA
CCCAGCGGCGCGACGCGCCACCACCGCCGAGACCGCGTCCGCCCCGCGAGCACAGAG
CCTCGCCTTTGCCGATCCTCTAGAGTCGAGATCCGCCGCCACCATGaccgagtacaagcccacggt
gcgcctcgccacccgcgacgacgtccccagggccgtacgcaccctcgccgccgcgttcgccgactaccccgccacgcgccacaccgtcga
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ATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAAT
AGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTC
CAAACTCATCAATGTATCTTATCATGTCTGGATCCGGGGGTACCGCGTCGAGAAGTTCC
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GCATACATTATACGAAGTTATGTCGAGATATCTAGACCCAGCTTTCTTGTACAAAGTTG
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ACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGG
TAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTA
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ACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGA
TAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCC
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AGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTC
GGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGT
CCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGG
GCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCT
GGCCTTTTGCTCACATGTT

Legend: Yellow highlighted: attL1 and attL2 gateway sites respectively
Red: EF1α-puro-polyA cassette

A12: Sequence of pAAAS-EF1α-puro-polyA donor plasmid

5’-
tcgcgcgtttcggtgatgacggtgaaaacctctgacacatgcagctcccggagacggtcacagcttgtctgtaagcggatgccgggagcagac
aagcccgtcagggcgcgtcagcgggtgttggcgggtgtcggggctggcttaactatgcggcatcagagcagattgtactgagagtgcaccat
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GGGAACGGTTAAGGAGCTGTGGAGGAGGCCTAGAGGGAATGGAGAGAATGAGGGAG
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ATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGAACCAAACGGCGCGCCGC
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AGGGACCGTCGTTAAACTCCCACTAACGTAGAACCCAGAGATCGCTGCGTTCCCGCCC
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GGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGA
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GTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTG
CCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGA
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TGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGAC
GCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTT
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CCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCA
AGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTG
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tgtctcatgagcggatacatatttgaatgtatttagaaaaataaacaaatagggttccgcgcacatttccccgaaaagtgccacctgacgtctaa
gaaaccattattatcatgacattaacctataaaaataggcgtatcacgaggccctttcgtc – 3’

Legend:
Purple: InFusion primers
Green: U5 and D3 recombineering primers respectively
Red: EF1α-puro-polyA cassette
Yellow highlighted: attB1 and attB2 sites respectively
Underlined: 5’ and 3’ homology arms respectively
Blue: pUC19 seq_F and seq_R respectively
Orange: ampicillin resistance

A13: Sequence of HTT synthetic plasmid

5’-
GCTGAGCGGCGCCGCGAGTCGGCCCGAGGCCTCCGGGGACTGCCGTGCCGGGCGGGA
GACaGCCATGGCGACCCTGGAAAAGCTGATGAAGGCaTTCGAGTCCCTCAAGTCCTTC
CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG
CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG
CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAG
CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCCGCCACCGCCGCCGC
CGCCGCCGCCGCCTCCTCAGCTTCCTCAGCCGCCGCCGCAGGCACAGCCGCTGCTGCC
TCAGCCGCAGCCGCCCCCGCCGCCGCCCCCGCCGCCACCCGGCCCGGCTGTGGCTGAG
GAGCCGCTGCAC -3’

Legend:
Red: cloning sites BlpI and BsgI respectively
Bold: 67-Q repeats
Underlined: CRISPR sites 4 and 3 respectively
Blue lower-case: PAM mutations

A14: Sequence of Amino acid sequences of three ALADIN isoforms recognized by the Proteintech anti-AAAS antibody and by a different monoclonal antibody (Sigma, as an example)

Q9NRG9-V1 (60kDa):
MCSLGLFPPPPPRGQVTLYEHNNELVTGSSYESPPPDFRGQWINLPVLQLTKDPLKTPGRLD
HGTRTAFIHHREQVWKRCINIWRDVGLFGVLNEIANSEEEVFEWVKTASGWALALCRWA
SSLHGSLFPHLSLRSEDLIAEFAQVTNWSSCCLRVFAWHPHTNKFAVALLDDSVRVYNASS
TIVPSLKHRLQRNVASLAWKPLSASVLAVACQSCILIWTLDPTSLSTRPSSGCAQVLSHPGH
TPVTSLAWAPSGGRLLSASPVDAAIRVWDVSTETCVPLPWFRGGGVTNLLWSPDGSKILA
TTPSAVFRVWEAQMWTCERWPTLSGRCQTGCWSPDGSRLLFTVLGEPLIYSLSFPERCGE
GKGCVGGAKSATIVADLSETTIQTPDGEERLGGEAHSMVWDPSGERLAVLMKGKPRVQD
GKPVILLFRTRNSPVFELLPCGIIQGEPGAQPQLITFHPSFNKGALLSVGWSTGRIAHIPLYFV
NAQFPRFSPVLGRAQEPPAGGGGSIHDLPLFTETSPTSAPWDPLPGPPPVLPHSPHSHL

Q9NRG9-V2 (56kDa):
MCSLGLFPPPPPRGQVTLYEHNNELVTGSSYESPPPDFRGQWINLPVLQLTKDPLKTPGRLD
HGTRTAFIHHREQVWKRCINIWRDVGLFGVLNEIANSEEEVFEWVKTASGWALALCRWA
SSLHGSLFPHLSLRSEDLIAEFAQVTNCTIVPSLKHRLQRNVASLAWKPLSASVLAVACQSC
ILIWTLDPTSLSTRPSSGCAQVLSHPGHTPVTSLAWAPSGGRLLSASPVDAAIRVWDVSTET
CVPLPWFRGGGVTNLLWSPDGSKILATTPSAVFRVWEAQMWTCERWPTLSGRCQTGCWS
PDGSRLLFTVLGEPLIYSLSFPERCGEGKGCVGGAKSATIVADLSETTIQTPDGEERLGGEA

HSMVWDPSGERLAVLMKGKPRVQDGKPVILLFRTRNSPVFELLPCGIIQGEPGAQPQLITF
HPSFNKGALLSVGWSTGRIAHIPLYFVNAQFPRFSPVLGRAQEPPAGGGGSIHDLPLFTETS
PTSAPWDPLPGPPPVLPHSPHSHL

F8VZ44 (46kDa):

MKLQTQKKRLRSEDLIAEFAQVTNWSSCCLRVFAWHPHTNKFAVALLDDSVRVYNASSTI
VPSLKHRLQRNVASLAWKPLSASVLAVACQSCILIWTLDPTSLSTRPSSGCAQVLSHPGHT
PVTSLAWAPSGGRLLSASPVDAAIRVWDVSTETCVPLPWFRGGGVTNLLWSPDGSKILAT
TPSAVFRVWEAQMWTCERWPTLSGRCQTGCWSPDGSRLLFTVLGEPLIYSLSFPERCGEG
KGCVGGAKSATIVADLSETTIQTPDGEERLGGEAHSMVWDPSGERLAVLMKGKPRVQDG
KPVILLFRTRNSPVFELLPCGIIQGEPGAQPQLITFHPSFNKGALLSVGWSTGRIAHIPLYFVN
AQFPRFSPVLGRAQEPPAGGGGSIHDLPLFTETSPTSAPWDPLPGPPPVLPHSPHSHL

Legend:

Yellow highlighted: Polyclonal (Proteintech) antibody binding sequence
Red: Possible monoclonal (Sigma) antibody binding sequence

Additional figures

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Genomic Location	Overlapping Gene(s)	Orientation	Query start	Query end	Length	Score	E-val	%ID
12:53307558-53307713 [Sequence]	AAAS	Reverse	473	524	52 [Sequence]	88.1	5e-16	100.00 [Alignment]
12:53314297-53314443 [Sequence]	AAAS	Reverse	182	230	49 [Sequence]	78.0	1e-12	100.00 [Alignment]
12:53320510-53320692 [Sequence]	AAAS	Reverse	42	100	61 [Sequence]	76.5	3e-12	77.05 [Alignment]
12:53308657-53309025 [Sequence]	AAAS	Reverse	311	382	123 [Sequence]	75.6	7e-12	46.34 [Alignment]
12:53321343-53321465 [Sequence]	AAAS	Reverse	1	41	41 [Sequence]	73.5	3e-11	100.00 [Alignment]
12:53309156-53309281 [Sequence]	AAAS	Reverse	271	312	42 [Sequence]	71.7	1e-10	100.00 [Alignment]
12:53309598-53309726 [Sequence]	AAAS	Reverse	229	271	43 [Sequence]	68.5	1e-09	97.67 [Alignment]
12:53314744-53314848 [Sequence]	AAAS	Reverse	150	184	35 [Sequence]	58.1	3e-06	91.43 [Alignment]
12:53308434-53308562 [Sequence]	AAAS	Reverse	348	394	47 [Sequence]	56.9	4e-13	80.85 [Alignment]
12:53315332-53315457 [Sequence]	AAAS	Reverse	93	134	42 [Sequence]	54.2	5e-05	76.19 [Alignment]
6:95630270-95630467 [Sequence]	RP11-374I15.1	Reverse	390	457	68 [Sequence]	50.9	2e-06	52.94 [Alignment]
12:53307976-53308191 [Sequence]	AAAS	Reverse	402	485	93 [Sequence]	50.6	7e-04	46.24 [Alignment]
12:53307845-53307928 [Sequence]	AAAS	Reverse	445	472	28 [Sequence]	49.1	0.002	100.00 [Alignment]
12:53308217-53308354 [Sequence]	AAAS	Reverse	393	444	52 [Sequence]	45.6	4e-13	55.77 [Alignment]
6:95630149-95630277 [Sequence]	RP11-374I15.1	Reverse	451	496	46 [Sequence]	28.3	2e-06	47.83 [Alignment]

Figure A15: BLAST results of the Q9NRG9 isoform against the human genome indicating 52.94% and 47.83% identities (in red arrows) to a processed pseudogene, RP11-374I15.1 on chromosome 6

List of figures and tables