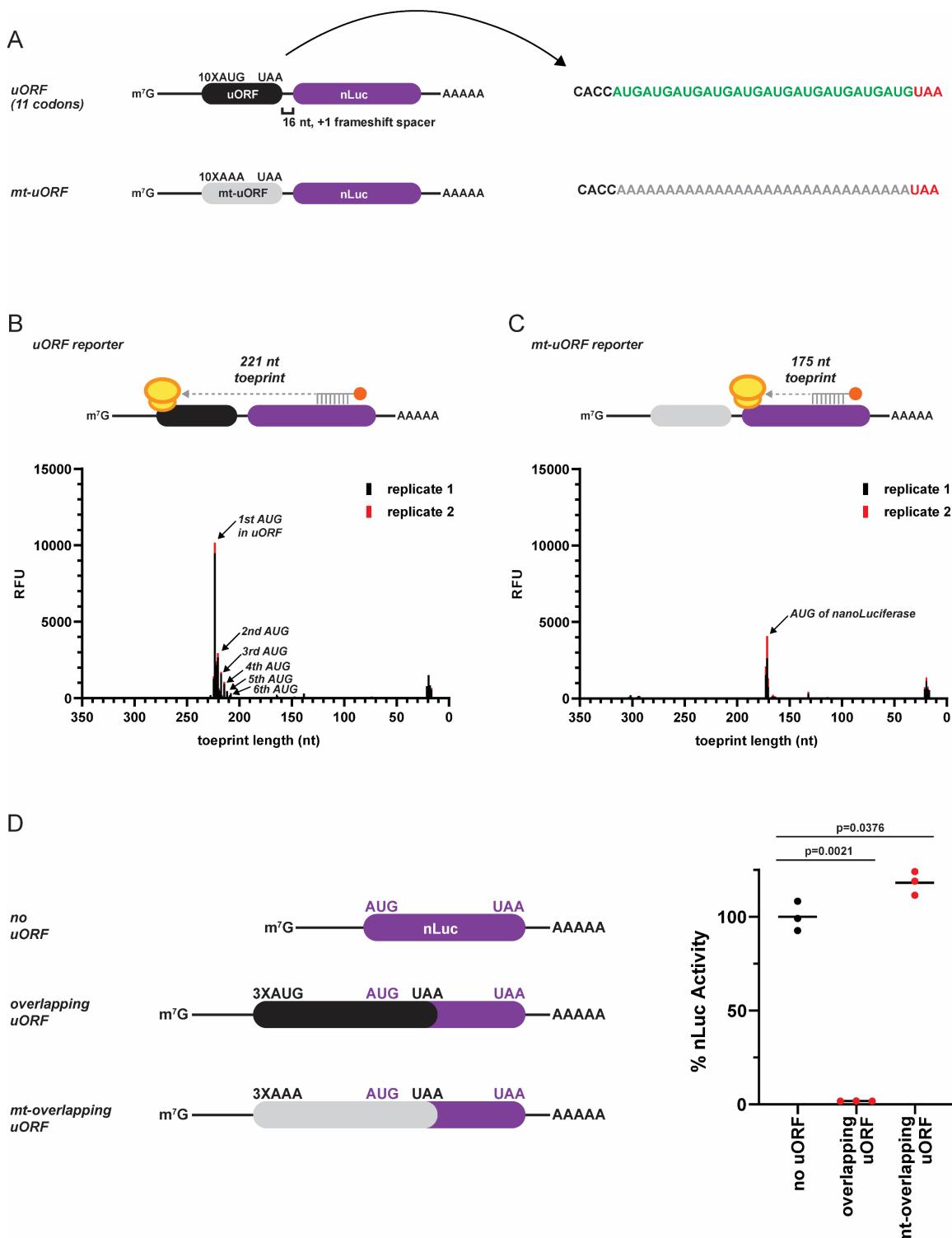
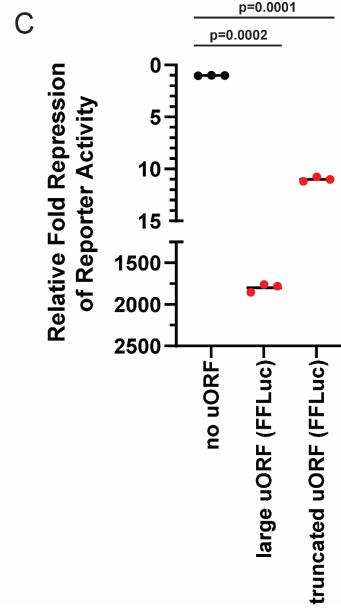
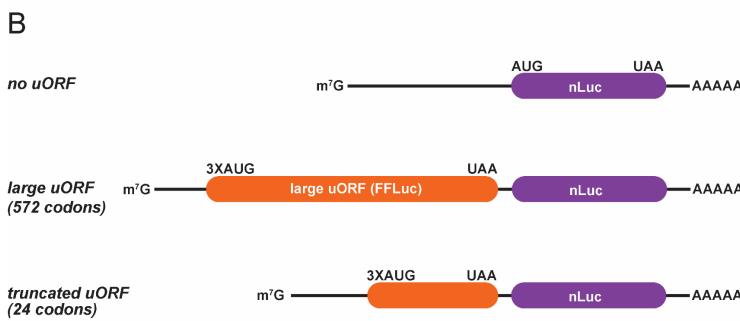
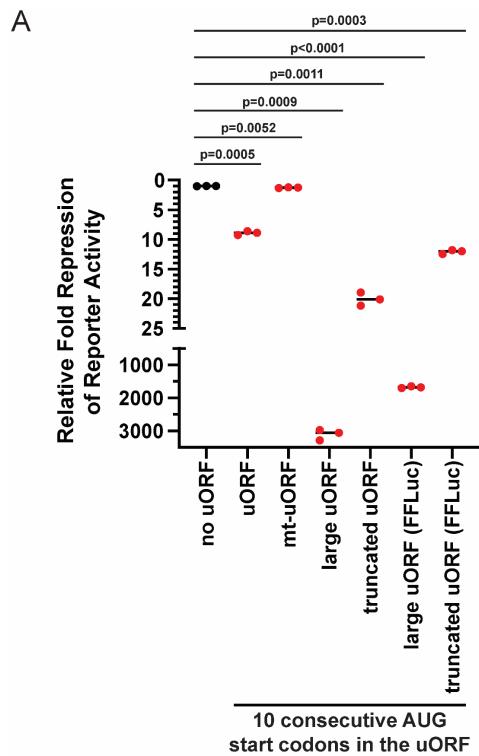


SUPPLEMENTAL MATERIAL



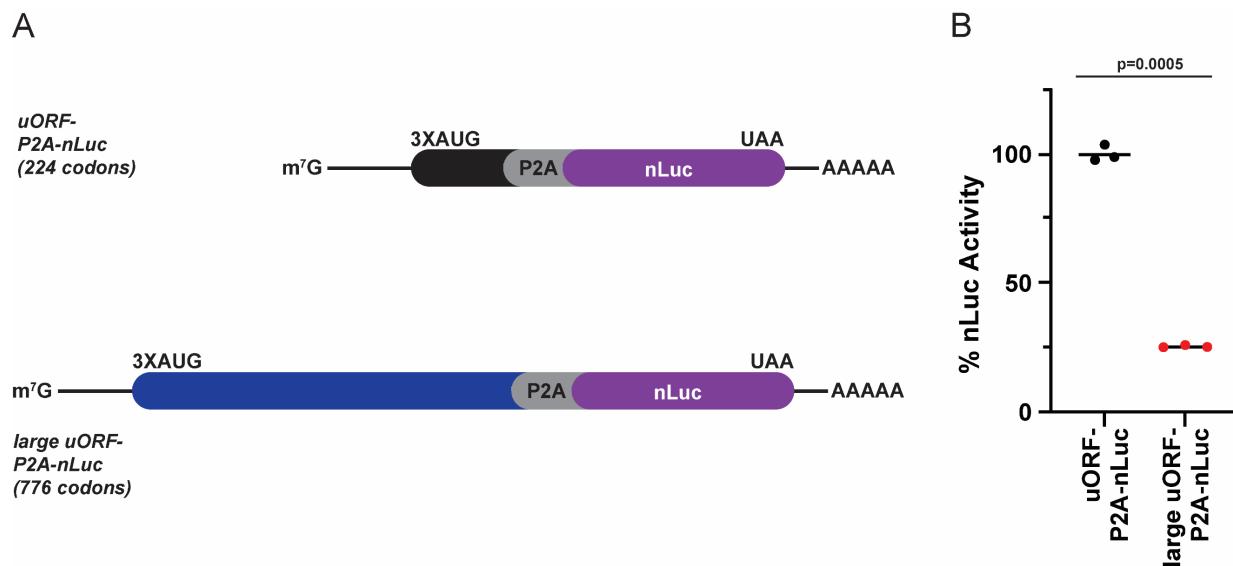
Supplemental Figure 1. Confirming the efficacy of designed uORFs to sequester all scanning initiation complexes *in vitro* and in HeLa cells. A) Design of re-initiation specific nanoLuciferase (nLuc) reporters harboring an uORF encoded by 10 consecutive AUG start

codons (uORF) or AAA codons (mt-uORF). A 16 nt spacer between the uORF and nLuc ORF allows specific detection of re-initiation. B-C) Ribosome toeprinting of lactimidomycin inhibited 80S ribosomes after start codon recognition on (B) small uORF nLuc reporter mRNA and (C) mutated uORF nLuc reporter mRNA from *in vitro* translation. Signal from duplicate samples is shown in black and red. D) Response of nLuc reporters that harbor an overlapping 3XAUG start codon uORF in HeLa cells. Here, the stop codon of the uORF (black) is downstream and out-of-frame of the start codon of nLuc (purple). Thus, active nLuc is only produced if a 43S PIC scans through the overlapping uORF (“leaky scanning”) and initiates at the start codon of nLuc. n=3 biological replicates. Bar represents the mean. Comparisons were made using a two-tailed unpaired *t*-test with Welch’s correction.



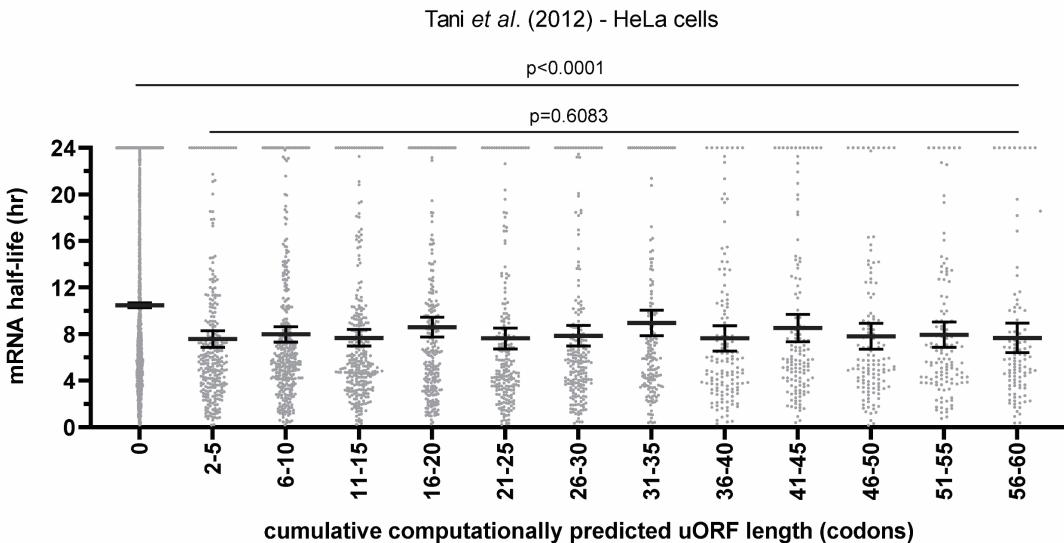
Supplemental Figure 2. Less translation re-initiation after large uORFs is not strictly uORF sequence specific. A) Response of nLuc reporters that harbor a small, mutant, large, or truncated uORF from *in vitro* translation. All uORFs had 10 consecutive AUG start codons as described in **Supplemental Figure 1A** (instead of the three AUG start codons in perfect Kozak

context in **Fig. 1**). Large and truncated uORFs using the FFLuc sequence are clearly labeled from those that used the original HT-GFP sequence. n=3 biological replicates. Bar represents the mean. Comparisons were made using a two-tailed unpaired *t*-test with Welch's correction. B) The large uORF sequence described in **Fig. 1** was switched from HT-GFP to Firefly Luciferase (FFLuc). Three AUG start codons in perfect Kozak context were used to trap all scanning initiation complexes at the uORF. C) Response of nLuc reporters that harbor a large (FFLuc) or truncated uORF (FFLuc) from *in vitro* translation. Similar repression and rescue were seen here with FFLuc as the large uORF as observed with HT-GFP sequence in **Fig. 2**. n=3 biological replicates. Bar represents the mean. Comparisons were made using a two-tailed unpaired *t*-test with Welch's correction.

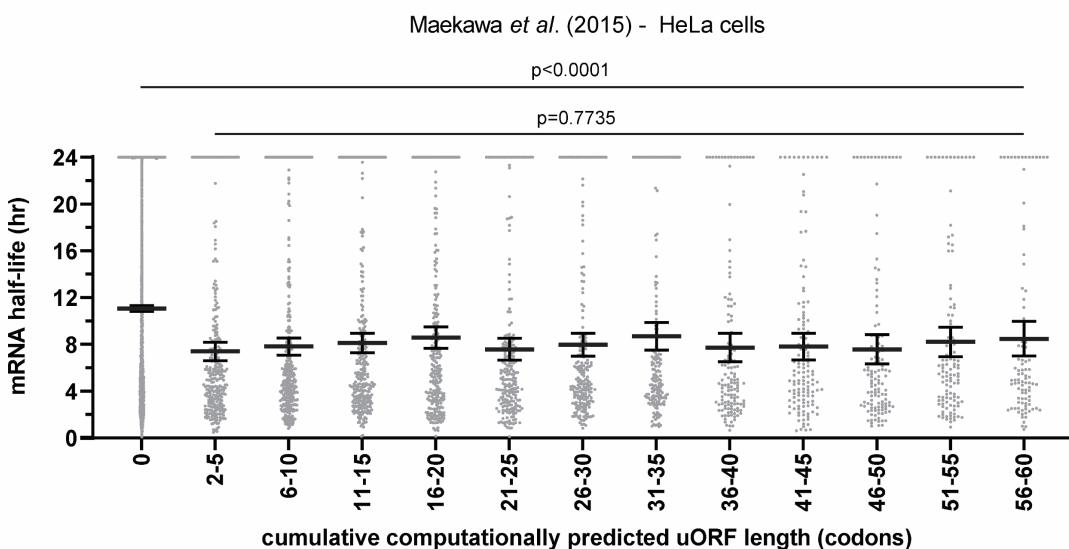


Supplemental Figure 3. Small and large uORF P2A fusion reporters to test translation efficiency *in vitro*. A) Insertion of the P2A “ribosome skipping motif” (gray) was used to assess the relative translation efficiency of reporters that harbored a 3XAUG start codon sequence without and with the large HT-GFP (blue) sequence upstream of the nLuc coding sequence (purple). B) Relative nLuc activity of the small and larger uORF P2A fusion reporters from *in vitro* translation. n=3 biological replicates. Bar represents the mean. Comparisons were made using a two-tailed unpaired *t*-test with Welch’s correction.

A



B



Supplemental Figure 4. uORF length has minimal effect on mRNA stability in cells. A-B)

Transcriptome-wide comparison of cumulative computationally predicted uORF length and mRNA half-life from Tani *et al.* (2012) (A) and Maekawa *et al.* (2015) (B). Bar represents the mean \pm 95% confidence interval. One-way Welch's ANOVA was used to compare between cumulative uORF length bins.

Sequences of reporters used in this study

The plasmid backbone, reporter name, and restriction sites are given for each construct. For reference, nanoLuciferase (nLuc) is always highlighted in pink. The multiple start sites for the uORF and single stop codon are highlighted in yellow and red, respectively. The HT-GFP sequence, FFLuc sequence and IRES sequences are highlighted in green, blue, and turquoise, respectively. The unstructured CAA repeats are in lowercase. The P2A sequence is highlighted in gray. The strong hairpins used upstream of the IRES sequences is in bold. For the pTet-Off All-In-One (pTet-Off AIO) plasmids, the tight TRE promotor is shown in purple and the intron is underlined.

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pcDNA3.1+/uORF nLuc (Sacl/XbaI)

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pcDNA3.1+/mt-uORF nLuc (Sacl/XbaI)

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pcDNA3.1+/10AUG uORF nLuc (Sacl/XbaI)

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pcDNA3.1+/10AAA mt-uORF nLuc (Sacl/XbaI)

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pcDNA3.1+/large uORF nLuc (Sacl/XbaI)

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pcDNA3.1+/truncated uORF nLuc (Sacl/XbaI)

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pcDNA3.1+/10AUG large uORF nLuc (Sacl/XbaI)

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pcDNA3.1+/large uORF (FFLuc) nLuc (Sacl/XbaI)

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 AGGATTGTCCTGAGCGGTGAAATGGGCTGAAAGATCGACATCCATGTCATCATCCGTATG
 AAGGTCTGAGCGCGACCAATGGGCCAGATCGAAAAAAATTGTTAAGGTGGTGTACCCCTGT
 GGATGATCATCACTTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGGGTTACG
 CCGAACATGATCGACTATTCCGGACGGCCGTATGAAGGCATGCCGTGTCGACGGAAA
 AAGATCACTGTAACAGGGACCCCTGGAACGGCAACAAAATTATCGACGAGCGCCTGATC
 AACCCCGACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGGCT
 GTGCGAACGCATTCTGGCGGACTACAAAGACCATGACGGTGATTATAAGATCATGACATC
 GATTACAAGGATGACGATGACAAGTAAGGCCCGACTCTAGA

pcDNA3.1+/truncated uORF (FFLuc) nLuc (Sacl/XbaI)

GAGCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAATACGACTCA
CTATAGGcaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaaca
aacaccatggtcaccatggtcaccatggtcGAAGATGCCAAAAACATTAAGAAGCTGCTGGCCTGGATA
GCACOtaaacaacaacaacaacaaATGGTCTTCACACTCGAAGATTGCTGGGACTGGCGACA
GACAGCCGGCTACAACCTGGACCAAGTCCTGAACAGGGAGGTGTCCAGTTGTTCA
GAATCTCGGGGTGCCGTAACTCCGATCCAAGGATTGCTCTGAGCGGTGAAAATGGGCT
GAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGGCGACCAAATGGCCA
GATCGAAAAAAATTAAAGGTGGTGTACCCCTGTTGATGATCATCACTTAAGGTGATCCTGC
ACTATGGCACACTGGTAATCGACGGGTTACGCCGAACATGATCGACTATTGCGACGGC
CGTATGAAGGCATGCCGTGTTGACGGCAAAAAGATCACTGTAACAGGGACCCGTGGA
ACGGCAACAAAATTATCGACGAGCGCCTGATCAACCCCCACGGCTCCCTGCTGTTCCGAG
TAACCATCAACGGAGTGACCGGCTGGCGCTGTGCGAACGCATTCTGGCGGACTACAAAG
ACCATGACGGTGATTATAAGATCATGACATCGATTACAAGGATGACGATGACAAGTAAGG
CCGCGACTCTAGA

pcDNA3.1+/10AUG large uORF (FFLuc) nLuc (Sacl/XbaI)

TGGGATGATCATCACTTAAGGTGATCCCTGCACATGGCACACTGGTAATCGACGGGGTTA
CGCCGAACATGATCGACTATTCGGACGGCGTATGAAGGCATGCCGTTCGACGGCA
AAAAGATCACTGTAACAGGGACCCTGTTGAACGGCAACAAATTATCGACGAGGCCCTGA
TCAACCCCACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGG
CTGTGCGAACGCATTCTGGCGACTACAAAGACCATGACGGTGATTATAAGATCATGACA
TCGATTACAAGGATGACGATGACAAGTAAGGCCCGACTCTAGA

pcDNA3.1+/overlapping uORF nLuc (Sacl/XbaI)

GAGCTCTGGCTAACTAGAGAACCCACTGCTACTGGCTATCGAAATTAAATACGACTCA
CTATAGGCaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaaca
aacaccatggtccccatggtcaccaatggcGTGAGCAAGGGGAGGGATGGTCTTCACACTCGAAGATTTC
GTTGGGGACTGGCGACAGACAGCCGGTACAACCTGGACCAAGTCCTGAACAGGGAGG
TGTGTCAGTTGTTAGAATCTCGGGGTGTCCGTAACTCCGATCCAAGGATTGTCCTG
AGCGGTGAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGC
GGCGACCAAATGGGCCAGATCGAAAAAAATTAAAGGTGGTGTACCTGTGGATGATCATC
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CGACTATTCGGACGGCGTATGAAGGCATGCCGTGTCACGGCAAAAGATCACTGT
AACAGGGACCCCTGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCAACCCCGACGG
CTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGCTGTGCGAACGCAT
TCTGGCGGACTACAAAGACCATGACGGTGATTATAAGATCATGACATCGATTACAAGGAT
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pcDNA3.1+/mt-overlapping uORF nLuc (Sacl/XbaI)

GAGCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAATACGACTCA
CTATAGGcaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaac
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TCACTTAAAGGTGATCCTGCACATGGCACACTGGTAATCGACGGGTTACGCCAACATG
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GTAACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAGCCCTGATCAACCCCGAC
GGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGGCTGTGCGAACG
CATTCTGGCGGACTACAAAGACCATGACGGTGATTATAAGATCATGACATCGATTACAAG
GATGACGATGACAAGTAAGGCCCGACTCTAGA

pcDNA3.1+/10AUG truncated uORF (FFLuc) nLuc (Sacl/XbaI)

CAAAGACCATGACGGTATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAG
TAAGGCCCGACTCTAGA

pcDNA3.1+/uORF-P2A-nLuc (Sacl/XbaI)

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TTCCGAGTAACCATCAACGGAGTGACCGGCTGGCGCTGTGCGAACGCATTCTGGCGGA
CTACAAAGACCATGACGGTATTATAAAGATCATGACATCGATTACAAGGATGACGATGAC
AAGTAAGGCCCGACTCTAGA

pcDNA3.1+/large uORF-P2A-nLuc (Sacl/XbaI)

GAGCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAATACGACTCA
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GTTGCACCGACCCATCGCTGCATTGCTCCAGACCTGATCGGTATGGCAAATCCGACAAA
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 GTGATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGTAAGGCCGCACTC
 TAGA

pcDNA3.1+/PV IRES no uORF nLuc (Sacl/XbaI)

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 TAAGGCCGCACTCTAGA

pcDNA3.1+/PV IRES uORF nLuc (Sacl/XbaI)_16 nt spacer

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pcDNA3.1+/PV IRES uORF nLuc (Sacl/XbaI)_115 nt spacer

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 CCCGTAACCTAGAACGACAATGTCCAAGTCAATAGGAGGGGTACAAACCAAGTACCA
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pcDNA3.1-D/EMCV IRES no uORF nLuc (Sacl/XbaI)

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 ACGGTGATTATAAGATCATGACATCGATTACAAGGATGACGATGACAAGTAAAGGGTCA
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pcDNA3.1-D/EMCV IRES uORF nLuc (Sacl/XbaI)_16 nt spacer

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pcDNA3.1-D/EMCV IRES uORF nLuc (Sacl/XbaI)_115 nt spacer

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pcDNA3.1-D/HCV no uORF nLuc (Sacl/XbaI)

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GACGGCAAAAGATCACTGTAACAGGGACCCTGTGGAACGGCAACAAAATTATCGACGAG
CGCCTGATCAACCCCGACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGC
TGGCGGCTGTCGAACCGATTCTGGCGGACTACAAAGACCATGACGGTGATTATAAGAT
CATGACATCGATTACAAGGATGACGATGACAAGTAAAGGGTCAAGACAATTCTGCAGATA
TCCAGCACAGTGGCGGCCGCTCGAGCTAGA

pcDNA3.1-D/HCV IRES uORF nLuc (Sacl/XbaI)_16 nt spacer

GAGCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAACGACTCA
CTATAGGGAGACCCAAGCTGGTGGGCGCGTGGTGGCGGCTGCAGCCGCCACCACGC
GCCCCGGCTAGTTAAGCTTGGTACCGAGCTCGGATCCCCGTGAGGAACTACTGTCTTCAGC
CGCAGAAAGCGCCTAGCCATGGCGTTAGTATGAGTGTCTGACAGCCTCCAGGCCCCCCCC
TCCCAGGAGAGCCATAGTGGTCTGCAGAACCGGTGAGTACACCGGAATTGCCGGGAAGA
CTGGGTCTTCTTGGATAAACCCACTCTATGCCGGCCATTGGCGTGCACCGCAAG

ACTGCTAGCCGAGTAGCGTTGGGTTGCGAAAGGCCTTGTGGTACTGCCTGATAGGGCGCT
TGCAGTGCCCCGGGAGGTCTCGTAGACCGTGCatcatgacgacgaaatctaaacctaaagaaaaaaa
caacaacaacaacaaaATGGTCTTCACACTCGAAGATTGCGTTGGGACTGGCGACAGACAGCCG
GCTACAACCTGGACCAAGTCCTGAACAGGGAGGTGTGTCAGTTGTTCAGAACTCGG
GGTGTCCCGTAACCTCGATCCAAAGGATTGTCCTGAGCGGTGAAAATGGGCTGAAGATCGA
CATCCATGTCATCATCCCCTGATGAGGTCTGAGCGGCACCAAATGGGCCAGATCGAAAAA
AATTTTAAGGTGGTGTACCCCTGATGATGATCATCACTTTAAGGTGATCCTGCACTATGGCA
CACTGGTAATCGACGGGTTACGCCAACATGATCGACTATTCGGACGGCCGTATGAAG
GCATCGCCGTGTCGACGGAAAAAGATCACTGTAACAGGGACCCCTGTTGGAACGGCAACAA
AAATTATCGACGAGCGCCTGATCAACCCCGACGGCTCCCTGCTGTTCCGAGTAACCATCAA
CGGAGTGACCGGCTGGCGCTGCGAACGCAATTCTGGCGGACTACAAAGACCATGACCG
GTGATTATAAGATCATGACATCGATTACAAGGATGACCGATGACAAGTAAAAGGGTCAAGA
CAATTCTGCAGATATCCAGCACAGTGGCGGCCGCTGAGTCTAGA

pcDNA3.1-D/HCV IRES uORF nLuc (Sacl/XbaI)_115 nt spacer

GAGCTCTGGCTAACTAGAGAACCCACTGCTACTGGCTATCGAAATTAAACGACTCA
CTATAGGGAGACCCAAGCTGGTTGGGCGCGTGGTGGCGCTGCAGCCGCCACCACGC
CCCCCGGCTAGTTAAGCTGGTACCGAGCTCGGATCCCTGTGAGGAACACTGTCTCA
CGCAGAAAGGCCCTAGCCATGGCGTTAGTATGAGTGTGTCGACGCCCTCAGGCC
TCCCAGGGAGAGCCATAGTGGTCTGCGAACCGGTGAGTACACCGGAATTGCC
CTGGGTCTTCTTGATAAACCCACTCTATGCCCGGCATTGGCGTGCCCCCGCAAG
ACTGCTAGCCGAGTAGCGTTGGTGGAAAGGCCTGTGGTACTGCCTGATAGGGCGCT
**TGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCatcatgagcacgaatccta
aaaccta
aaa**
caac
aacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaacaaca
GGCGACAGACAGCCGGCTACAACCTGGACCAAGTCCTGAACAGGGAGGTGTG
CCAGTTGTTTCAGAATCTGGGTGTCGTAACCTCGATCCAAGGATTG
TCCTGAGCGGTGAAAA
TGGGCTGAAGATCGACATCCATGT
CATCCCCTATGAAGG
GTCTGAGCGGC
GACCAAAT
GGGCCAGATCGAAAAAA
TTTAAGGTGGT
TACCTG
GGATGATCAT
CACTTAA
AGGTG
ATCCTG
CACT
ATGG
CACACT
GGTA
ATCG
ACGG
GGTT
ACGCC
GAAC
ATG
ATCG
ACT
ATTC
G
GACGG
CCGT
ATGA
AGGC
CATCG
CCGT
TTCG
ACGG
CAAA
AGAT
CACT
GTAA
CAGGG
ACCC
TCC
GAG
TAAC
CCAT
AACGG
AGTG
ACCG
GGCT
GGCG
GCTG
CGAAC
GCATT
CTGG
CGAC
TAC
AAA
AGG
ACCAT
GACGG
GTGATT
TAA
AGAT
CATG
ACAT
CGATT
ACA
AGG
ATG
ACG
ATG
ACA
AGT
CTAG
A

pcDNA3.1-D/CrPV IGR IRES no uORF nLuc (Sacl/XbaI)

GAGCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAACGACTCA
CTATAGGGAGACCCAAGCTGGTTGGGCGCGTGGCTGCAGCCGCCACCAACGC
GCCCCGGCTAGTTAAGCTTGGTACCGAGCTCGGATCCAGTACCCTCACCAAAGCAAAAT
GTGATCTTGCTTGTAAATACAATTGAGAGGTTAATAAATTACAAGTAGTGCTATTTGTA
TTTAGGTTAGCTATTAGCTTACGTTCCAGGATGCCTAGTGGCAGCCCCACAATATCCAG
GAAGCCCTCTGCGGTTTCAGATTAGGTAGTCGAAAAACCTAAGAAATTACCTGCTAC
ATTCAAGATACCATGGTCTTCACACTCGAAGATTGTTGGGACTGGCGACAGACAGCC
GGCTACAAACCTGGACCAAGTCCTGAACAGGGAGGTGTGTCAGTTGTTAGAAATCTCG
GGGTGTCGTAACTCCGATCCAAAGGATTGTCCTGAGCGGTGAAAATGGGCTGAAGATCG
ACATCCATGTCATCATCCGTATGAAGGTCTGAGCGGCACCAAATGGGCCAGATCGAAA
AAATTGTAAGGTGGTGTACCCGTGGATGATCATCACTTAAGGTGATCCTGCACTATGGC
ACACTGGTAATCGACGGGTTACGCCAACATGATCGACTATTGCGACGGCGTATGAA
GGCATCGCCGTGTCAGGGAAAAAGATCAGTAAACAGGGACCCGTGGAACCGAAC

AAAATTATCGACGAGCGCCTGATCAACCCCGACGGCTCCCTGCTGTTCCGAGTAACCATCA
ACGGAGTGACCGGCTGGCGGCTGTGCGAACGCATTCTGGCGGACTACAAAGACCATGAC
GGTGTATTATAAAGATCATGACATCGATTACAAGGATGACGATGACAAGTAAAGGGTCAAG
ACAATTCTGCAGATATCCAGCACAGTGGCGGCCGCTCGAGTCTAGA

pcDNA3.1-D/CrPV IGR IRES uORF nLuc (Sacl/XbaI)_16 nt spacer

GAGCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAATACGACTCA
CTATAGGGAGACCCAAGCTGGTGGGCGCGTGGCTGCAGCCGCCACCACGC
CCCCCGGCTAGTTAAGCTTGGTACCGAGCTCGATCCAGTACCCCTCACCAAAGCAAAAAT
GTGATCTTGCTTGTAAATACAATTAGAGGTTAATAAATTACAAGTAGTGCTATTGGTA
TTTAGGTTAGCTATTAGCTTACGTTCCAGGATGCCTAGTGGCAGCCCCACAATATCCAG
GAAGCCCTCTGCGGTTTCAGATTAGGTAGTCGAAAAACCTAAGAAATTACCTGCTAC
ATTCAAGATAC**CACatggtaccatggtaccatggtcaaacaacaacaacaacaa**ATGGTCTTACACTCGAA
GATTTCTGGGACTGGCGACAGACAGCCGGCTACAACCTGGACCAAGTCCTGAACAG
GGAGGTGTGTCAGTTGTTAGAATCTGGGGTGTCCGTAACCTCGATCCAAAGGATTG
TCCTGAGCGGTGAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGGTCT
GAGCGGCGACCAAATGGGCCAGATCGAAAAAAATTAAAGGTGGTACCCGTGGATGA
TCATCACTTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGGGTTACGCCAAC
ATGATCGACTATTCGGACGGCGTATGAAGGCATGCCGTGTTGACGGCAAAAGATC
ACTGTAACAGGGACCCGTGGAACGGCAACAAAATTATCGACGAGCGCCTGATCAACCCC
GACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTGACCGGCTGGGGCTGTGCGA
ACGCATTCTGGCGGACTACAAAGACCATGACGGTGATTATAAGATCATGACATCGATTAC
AAGGATGACGATGACAAGTAAAAGGGTCAAGACAATTCTGCAGATATCCAGCACAGTGGC
GGCCGCTGAGTCTAGA

pcDNA3.1-D/CrPV IGR IRES uORF nLuc (Sacl/XbaI)_115 nt spacer

pTet-Off AIO/no uORF nLuc (MluI/XbaI)

ACGCGTTgctcgagcacttggccgcgaatcgatatgtcgagttactccatatcagtatcgatagagaacgtatgtcgagttactccat
atcagtatcgatagagaacgtatgtcgagttactccatatcagtatcgatagagaacgtatgtcgagttactccatatcagtatcgatagagaacgtat
gtcgagttactccatatcagtatcgatagagaacgtatgtcgagttactccatatcagtatcgatagagaacgtatgtcgagttactccatatcagt
qataqagaacgtatotcqaqgtaaqcgtatacqataqaaqcctatataaqcaGAGCTCTGGCTAACTAGAGAA

CCCACTGCTTACTGGCTTATCGAAATTAAATACGACTCACTATAGGCaacaacaacaacaacaacaac
aac
CGTTGGGGACTGGCGACAGACAGGCCGGTACAACCTGGACCAAGTCCTGAACAGGGAG
GTGTGTCAGTTGTTCAGAATCTCGGGGTGTCGTAACTCCGATCCAAGGATTGTCCT
GAGCGGTGAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCCTATGAAGGCTGAG
CGGCGACCAAATGGGCCAGATCGAAAAAAATTAAAGGTGGTGTACCCCTGTGGATGATCAT
CACTTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGGGTTACGCCAACATGA
TCGACTATTCGGACGGCCGTATGAAGGCATGCCGTGTCGACGGCAAAAGATCACTG
TAACAGGGACCCCTGTGGAACGGCAACAAAATTATCGACGTAAGTATCAAGGTTACAAGACA
GGTTTAAGGAGACCAATAGAAACTGGGCTTGTGAGACAGAGAAGACTCTTGCCTTCTGA
TAGGCACCTATTGGTCTTACTGACATCCACTTGCCTTCTCCACAGGAGCGCTGATC
AACCCCGACGGCTCCCTGCTGTTCCGAGTAACCACGAGTGACCCGGCTGGCGGCT
GTGCGAACGCATTCTGGCGGACTACAAAGACCATGACGGTATTATAAGATCATGACATC
GATTACAAGGATGACGATGACAAGTAAGGCCGCGACTCTAGA

pTet-Off AIO/no uORF nLuc + 3' UTR intron (MluI/PfIMI)

pTet-Off AIO/uORF nLuc (MluI/XbaI)

CACTGGTAATCGACGGGGTACGCCAACATGATCGACTATTCGGACGGCCGTATGAAG
GCATCGCCGTTCGACGGAAAAAGATCACTGTAACAGGGACCCTGTGGAACGGCAACA
AAATTATCGACGTAAAGTATCAAGGTTACAAGACAGGTTAAGGAGACCAATAGAAACTGGG
CTTGTGAGACAGAGAAGACTCTGCGTTCTGATAGGCACCTATTGGTCTTACTGACATC
CACTTGCCTTCTCTCCACAGGAGCGCCTGATCAACCCCCGACGGCTCCCTGCTGTTCCG
AGTAACCATCAACGGAGTGACCGGCTGGCGGCTGTGCGAACGCAATTCTGGCGGACTACAA
AGACCATGACGGTGATTATAAAGATCATGACATCGATTACAAGGATGACCGATGACAAGTAA
GGCCGCGACTCTAGA

pTet-Off AIO/large uORF nLuc (MluI/XbaI)

CAAGGTTACAAGACAGGTTAAGGAGACCAATAGAACTGGGCTTGTGAGACAGAGAAG
ACTCTTGCCTTCTGATAGGCACCTATTGGTCTTAAGTACATCCACTTGCCCTTCTCTCCA
CAGGAGCGCCTGATCAACCCCCGACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAGTG
ACCGGCTGGCGGCTGTGCGAACGCATTCTGGCGGACTACAAAGACCATGACGGTGATTAT
AAAGATCATGACATCGATTACAAGGATGACGATGACAAGTAAGGCCGCGACTCTAGA

pTet-Off AIO/HP + no uORF nLuc (MluI/XbaI)

pTet-Off AIO/HP + no uORF nLuc + 3' UTR intron (MluI/PfIMI)

ACAGAGAAGACTCTGCGTTCTGATAGGCACCTATTGGTCTACTGACATCCACTTGCCT
TTCTCTCCACAGCCCCATTATGTGG

pTet-Off AIO/HP + uORF nLuc (MluI/XbaI)

ACGCGTTgctcgagcacttggccgcgaatcgatatgtcgagttactccatatcagtatagagaacgtatgtcgagttactccct
 atcagtatagagaacgtatgtcgagttactccatatcagtatagagaacgtatgtcgagttactccatatcagtatagagaacgtat
 gtcgagttactccatatcagtatagagaacgtatgtcgagttatccatatcagtatagagaacgtatgtcgagttactccatatcagt
 atagagaacgtatgtcgaggtaggcgttacggggaggcctataagca**GAGCTC**acatttgctctgacacaactgttt
 actagcaacccaaacagacacc**TTGGGGCGCGTGGTGGCGCTGCAGCCGCCACCACGCGCCCC**
GGTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAATACGACTCACTATA
 GGacacc
 atggtcaccatggtcaccatggtc**aa**acaacaacaacaaca**ATGGTCTTCACACTCGAAGATTCTGTTGGG**
GACTGGCGACAGACAGCCGGCTACAACCTGGACCAAGTCCTGAAACAGGGAGGTGTGTC
CAGTTTGTTCAGAATCTCGGGGTGTCGTAACCTCGATCCAAGGATTGTCCCTGAGCGGGT
GAAAATGGGCTGAAGATCGACATCCATGTCATCATCCGTATGAAGGTCTGAGCGGGCAC
CAAATGGGCCAGATCGAAAAAATTAAAGGTGGTACCCGTGGATGATCATCACTTTAA
GGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTACGCCAACATGATCGACTA
TTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTGTAACAGG
GACCCCTGGAACGGCAACAAAATTATCGACGTAAGTATCAAGGTTACAAGACAGGTTAA
GGAGACCAATAGAAAATGGCTTGTGAGACAGAGAAGACTCTTGCCTTCTGATAGGCA
CCTATTGGTCTTACTGACATCCACTTGCCTTCTCCACAGGAGCGCCTGATCAACCCC
GACGGCTCCCTGCTGTTCCAGTAACCGAGCTGGAGTACCGGCTGGCGCTGTGCGA
ACGCATTCTGGCGGACTACAAAGACCATGACGGTATTATAAGATCATGACATCGATTAC
AAGGATGACGATGACAAGTAAGGCCGCGACTCTAGA

pTet-Off AIO/HP + large uORF nLuc (MluI/XbaI)

ACGCGTTgctcgagcacttggccgcgaatcgatatgtcgagttactccatatcagtatagagaacgtatgtcgagttactccct
 atcagtatagagaacgtatgtcgagttactccatatcagtatagagaacgtatgtcgagttactccatatcagtatagagaacgtat
 gtcgagttactccatatcagtatagagaacgtatgtcgagttatccatatcagtatagagaacgtatgtcgagttactccatatcagt
 atagagaacgtatgtcgaggtaggcgttacggggaggcctataagca**GAGCTC**acatttgctctgacacaactgttt
 actagcaacccaaacagacacc**TTGGGGCGCGTGGTGGCGCTGCAGCCGCCACCACGCGCCCC**
GGTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAAATACGACTCACTATA
 GGacacc
 tggtcaccatggtcaccatggtc**GCAGAAATCGGTACTGGCTTCCATTGACCCCCATTATGTGAA**
GTCCTGGCGAGCGCATGCACTACGTCATGTTGGTCCCGCGATGGCACCCCTGTGCT
GTTCTGCACGTAACCCGACCTCCTCCTACGTGTGGCGAACATCATCCCGATGTTGC
ACCGACCCATCGCTGCATTGCTCCAGACCTGATCGGTATGGCAAATCCGACAAACAGA
CCTGGGTTATTCTTGACGACCACGTCGCTTATGGATGCCATCGAAGCCCTGGGT
CTGGAAAGAGGTGCTGCTGGTCATTACGACTGGGCTCCGCTGGTTCCACTGGCC
AAGCGCAATCCAGAGCGCGTAAAGGTATTGCATTATGGAGTTCATCCGCCATCCGA
CCTGGGACGAATGCCAGAATTGCCCCGAGACCTCCAGGCCTCCGCACCACCGAC
GTCGGCCGCAAGCTGATCATCGATCAGAACGTTTATCGAGGGTACGCTGCCGATGGGT
GTCGTCGCCCGCTGACTGAAAGTCGAGATGGACCAATTACCGCGAGCCGTTCTGAATCCT
GTTGACCGCGAGCCACTGTGGCGCTCCAAACGAGCTGCCAATGCCGGTGAGCCAGC
GAACATCGCGCTGGTCGAAGAATACATGGACTGGCTGCACCAAGTCCCCGTCCCGAA
GCTGCTGTTCTGGGGCACCCCAGGCGTTCTGATCCCACCCGGCGAAGCCGCTCGCCTGG
CCAAAAGCCTGCTTAACGCAAGGCTGTGGACATCGGCCGGGTCTGAATCTGCTGCAAG
AAGACAACCCGGACCTGATCGGCAGCGAGATCGCGCGTGGCTGTCGACGCTCGAGATT
TCCGGCCACCGGTGCAACCTTGGTACCGCGGGGGATCCACCGGTGCAACCTT
GGTAGCAAGGGCGAGGAGCTGTTACCGGGGGTGGTGCCTACCTGGTCAGCTGGACG
GCGACGTAAACGCCACAAGTTCAGCGTGTCCGGCGAGGGCGATGCCACCTAC

GGCAAGCTGACCCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCCAC
CCTCGTGACCACCCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAA
GCAGCACGACTTCTCAAGTCCGCCATGCCCAGGGCTACGTCCAGGAGCGCACCACATCTT
CTTCAAGGACGACGGCAACTACAAGACCCCGCGAGGTGAAGTTCGAGGGCGACACCC
TGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGG
CACAAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGA
ACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCG
CCGACCACTACCAGCAGAACACCCCCCATCGGCGACGGCCCCGTGCTGCTGCCGACAAC
CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCAACGAGAACCGCGATCACATG
GTCCTGCTGGAGTTCTGTGACCGCCGCCGGATCACTCTGGCATGGACGAGCTGTACAA
GtaacaacaacaacaacaacaaATGGTCTTCACACTCGAAGATTCTGGGGACTGGCGACAGACA
GCCGGCTACAACCTGGACCAAGTCTTGAAACAGGGAGGTGTGCTCAGTTGTTCGAAT
CTCGGGGTGTCCGTAACCTCCGATCCAAGGATTGTCTGAGCGGTGAAAATGGGCTGAAG
ATCGACATCCATGTCATCATCCCGTATGAAGGTCTGAGCGGCGACCAAATGGGCCAGATC
GAAAAAAATTAAAGGTGGTGTACCCCTGTTGATGATCATCACTTAAGGTGATCCTGCAC
TGGCACACTGGTAATCGACGGGTTACGCCAACATGATCGACTATTCTGGACGGCCGTA
TGAAGGCATGCCGTGTCGACGGCAAAAGATCACTGTAACAGGGACCCGTGGAACGG
CAACAAAATTATCGACGTAAGTATCAAGGTTACAAGACAGGTTAAGGAGACCAATAGAAA
CTGGGCTTGTGAGACAGAGAAGACTCTGCGTTCTGATAGGCACCTATTGGTCTTACTG
ACATCCACTTTGCCCTTCTCTCCACAGGAGCGCCTGATCAACCCCGACGGCTCCCTGCTGT
TCCGAGTAACCATCAACGGAGTGACCGGCTGGCGCTGTGCGAACGCATTCTGGCGGAC
TACAAAGACCATGACGGTGATTATAAGATCATGACATCGATTACAAGGATGACGATGACA
AGTAAGGCCGCGACTCTAGA

pTet-Off AIO/no uORF nLuc + 3' UTR intron (MluI/PfIMI)_stop mutated

pTet-Off AIO/uORF nLuc (MluI/XbaI) stop mutated

pTet-Off AIO/large uORF nLuc (MluI/XbaI)_stop mutated

GCCCTGAGCAAAGACCCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCTGACC
GCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGtaccaacaacaacaacaaaATGGTCT
TCACACTCGAAGATTCTGTTGGGGACTGGCGACAGACAGCCGGCTACAACCTGGACCAAG
TCCTTGAAACAGGGAGGTGTGTCAGTTTCAAGATCTCGGGGTGTCGTAACCTCGAT
CCAAGGATTGTCTGAGCGGGCACAAATGGGCTGAAGATCGACATCCATGTCATCATCCC
GTATGAAGGTCTGAGCGGGCACAAATGGGCCAGATCGAAAAAAATTAAAGGTGGTGTAC
CCTGTGGATGATCATCACTTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGGGG
TTACGCCGAACATGATCGACTATTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACG
GCAAAAAGATCACTGTAACAGGGACCCCTGGAACGGCAACAAAATTATCGACGTAAGTAT
CAAGGTTACAAGACAGGTTAAGGAGACCAATAGAAACTGGGCTTGTGAGACAGAGAAG
ACTCTTGCCTTCTGATAGGCACCTATTGGCTTACTGACATCCACTTGCCTTCTCTCCA
CAGGAGCGCCTGATCAACCCGACGGCTCCCTGCTGTTCCGAGTAACCATCACGGAGTG
ACCGGCTGGCGGCTGTGCGAACGCATTCTGGCGGACTACAAAGACCATGACGGTGATTAT
AAAGATCATGACATCGATTACAAGGATGACGATGACAAGTAAGGCCGCGACTCTAGA