

# Extracellular ligand-responsive translational regulation of synthetic mRNAs using engineered receptors

## Supporting Information

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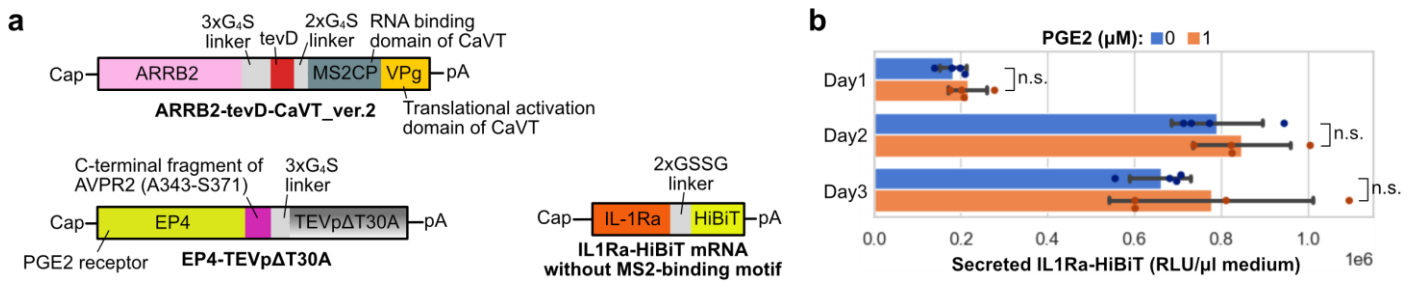
## Supplementary Figure

- Supplementary Figure S1 | Time course of IL-1Ra secretion from cells transfected with the mRNA lacking an MS2-binding motif.

## Supplementary Materials and Methods

- Full sequences of template DNAs for in vitro transcription.

## Supplementary Figures



### Supplementary Figure S1 | Time course of IL-1Ra secretion from cells transfected with the mRNA lacking an MS2-binding motif.

(a) Schematic diagram of mRNAs to check IL1Ra-HiBiT translation from a conventional mRNA. (b) Time course of IL1Ra-HiBiT secretion. HuH-7 cells were seeded onto a 96-well plate. One day after cell seeding, the medium was replaced with medium containing 0 or 1  $\mu\text{M}$  of PGE2. After the medium replacement, cells were transfected with IL1Ra-HiBiT (20 ng/well), Nluc (1 ng/well), ARR2B2-tevD-CaVT\_ver.2 (70 ng/well), and EP4-TEVp $\Delta$ T30A (10 ng/well) mRNAs. The medium was collected at the indicated time points. The bar graph shows HiBiT luminescence in 50  $\mu\text{l}$  of medium samples (mean  $\pm$  SD, n = 4).

## Supplementary Materials and Methods

### Full sequences of template DNAs for in vitro transcription

- **2xScMS2(C)-Luc2**

T7 promoter (for CleanCap AG Reagent): 11-30

Stabilized 2xMS2-binding motif (C-variant): 51-117

Kozak sequence (including start codon): 125-133

Luc2: 134-1780

Stop codon: 1781-1783

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[CAGTGAATTGTAATACGACTCACTATAAGGTCAGATCCGCTAGCGGATCCGGGAGCAGGTGAGGATCACC
CATCTGCCACGAGCGAGGTGAGGATCACCCATCTCGCTCGTGTCCACCGGTCGCCACCATGGAAGATG
CCAAAACATTAAGAAGGGCCCAGCGCCATTCTACCCACTCGAAGACGGGACCGCCGGCGAGCAGCTGC
ACAAAGCCATGAAGCGCTACGCCCTGGTGCCCGGCACCATCGCCTTTACCGACGCACATATCGAGGTGGA
CATTACCTACGCCGAGTACTTCGAGATGAGCGTTCGGCTGGCAGAAGCTATGAAGCGCTATGGGCTGAATA
CAAACCATCGGATCGTGGTGTGCAGCGAGAATAGCTTGCAGTTCTTCATGCCCGTGTGGGTGCCCTGTTC
ATCGGTGTGGCTGTGGCCCCAGCTAACGACATCTACAACGAGCGCGAGCTGCTGAACAGCATGGGCATCA
GCCAGCCCACCGTCGTATTCGTGAGCAAGAAAGGGCTGAAAAGATCCTCAACGTGCAAAAGAAGCTAC
CGATCATAAAAAGATCATCATCATGGATAGCAAGACCGACTACCAGGGCTTCCAAAGCATGTACACCTTC
GTGACTTCCCATTTGCCACCCGGCTTCAACGAGTACGACTTCGTGCCCGAGAGCTTCGACCCGGGACAAAA
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CGCTTGTGTCCGATTCAGTCATGCCCGGACCCCATCTTCGGCAACCAGATCATCCCCGACACCGCTATCCT
CAGCGTGGTGCCATTTACCACGGCTTCGGCATGTTACCACGCTGGGCTACTTGATCTGCGGCTTTTCGGG
TCGTGCTCATGTACCGCTTCGAGGAGGAGCTATTCTTGCGCAGCTTGCAAGACTATAAGATTCAATCTGCC
CTGCTGGTGCCACACTATTTAGCTTCTTCGCTAAGAGCACTCTCATCGACAAGTACGACCTAAGCAACTT
GCACGAGATCGCCAGCGGCGGGGCGCCGCTCAGCAAGGAGGTAGGTGAGGCCGTGGCCAAACGCTTCCA
CCTACCAGGCATCCGCCAGGGCTACGGCCTGACAGAAAACAACCAGCGCCATTCTGATCACCCCCGAAGGG
GACGACAAGCCTGGCGCAGTAGGCAAGGTGGTGCCCTTCTTCGAGGCTAAGGTGGTGGACTTGGACACC
GGTAAGACACTGGGTGTGAACCAGCGCGGCGAGCTGTGCGTCCGTGGCCCCATGATCATGAGCGGCTACG
TTAACAACCCCGAGGCTACAAACGCTCTCATCGACAAGGACGGCTGGCTGCACAGCGGCGACATCGCCTA
CTGGGACGAGGACGAGCACTTCTTCATCGTGGACCGGCTGAAGAGCCTGATCAAATACAAGGGCTACCAG
GTAGCCCCAGCCGAACCTGGAGAGCATCCTGCTGCAACACCCCAACATCTTCGACGCCGGGGTCGCCGGCC
TGCCCGACGACGATGCCGGCGAGCTGCCCGCCGAGTCGTGCTGCTGGAACACGGTAAAACCATGACCG
AGAAGGAGATCGTGGACTATGTGGCCAGCCAGGTTACAACCGCCAAGAAGCTGCGCGGTGGTGTGTTGTGTT
CGTGGACGAGGTGCCTAAAGGACTGACCGGCAAGTTGGACGCCCGCAAGATCCGCGAGATTCTCATTAAG
GCCAAGAAGGGCGGCAAGATCGCCGTGTAATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCT
TCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA]
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▪ **1xMS2(U)site2-Luc2**

T7 promoter: 11-30

MS2-binding motif (U-variant): 55-73

Kozak sequence (including start codon): 98-106

Luc2: 107-1753

Stop codon: 1754-1756

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[CAGTGAATTGTAATACGACTCACTATAGGGCGAATTAAGAGAGAAAAGAAGAGTACATGAGGATTACCCA
TGTAAGAAGAAATATAAGACACCCGGTTCGCCACCATGGAAGATGCCAAAACATTAAGAAGGGCCAGCGC
CATTCTACCCACTCGAAGACGGGACCGCCGGCGAGCAGCTGCACAAAGCCATGAAGCGCTACGCCCTGGT
GCCCCGGCACCATCGCCTTTACCGACGCACATATCGAGGTGGACATTACCTACGCCGAGTACTTCGAGATGA
GCGTTTCGGCTGGCAGAAGCTATGAAGCGCTATGGGCTGAATACAAACCATCGGATCGTGGTGTGCAGCGA
GAATAGCTTGCAGTTCTTCATGCCCCTGTTGGGTGCCCTGTTTCATCGGTGTGGCTGTGGCCCCAGCTAACG
ACATCTACAACGAGCGCGAGCTGCTGAACAGCATGGGCATCAGCCAGCCCACCGTCGTATTTCGTGAGCAA
GAAAGGGCTGCAAAGATCCTCAACGTGCAAAGAAGCTACCGATCATACAAAAGATCATCATATGGAT
AGCAAGACCGACTACCAGGGCTTCCAAAGCATGTACACCTTCGTGACTTCCCATTTGCCACCCGGCTTCA
ACGAGTACGACTTCGTGCCCGAGAGCTTCGACCGGGACAAAACCATCGCCCTGATCATGAACAGTAGTGG
CAGTACCGGATTGCCCAAGGGCGTAGCCCTACCGCACCGCACCGCTTGTGTCCGATTTCAGTCATGCCCGCG
ACCCCATCTTCGGCAACCAGATCATCCCCGACACCGCTATCCTCAGCGTGGTGCCATTTACCACGGCTTC
GGCATGTTACCCACGCTGGGCTACTTGATCTGCGGCTTTCGGGTCGTGCTCATGTACCGCTTCGAGGAGGA
GCTATTCTTGCGCAGCTTGCAAGACTATAAGATTCAATCTGCCCTGCTGGTGCCACACTATTTAGCTTCTT
CGCTAAGAGCACTCTCATCGACAAGTACGACCTAAGCAACTTGCACGAGATCGCCAGCGGGCGGGCGCCC
CTCAGCAAGGAGGTAGGTGAGGCCGTGGCCAAACGCTTCCACCTACCAGGCATCCGCCAGGGCTACGGC
CTGACAGAAACAACCAGCGCCATTCTGATCACCCCGAAGGGGACGACAAGCCTGGCGCAGTAGGCAAG
GTGGTGCCCTTCTTCGAGGCTAAGGTGGTGGACTTGGACACCGGTAAGACACTGGGTGTGAACCAGCGC
GGCGAGCTGTGCGTCCGTGGCCCCATGATCATGAGCGGCTACGTTAACAACCCCGAGGCTACAAACGCTC
TCATCGACAAGGACGGCTGGCTGCACAGCGGGCAGATCGCCTACTGGGACGAGGACGAGCACTTCTTCAT
CGTGGACCGGCTGAAGAGCCTGATCAAATACAAGGGCTACCAGGTAGCCCCAGCCGAACCTGGAGAGCAT
CCTGCTGCAACACCCCAACATCTTCGACGCCGGGGTCGCCGGCCTGCCCGACGACGATGCCGGCGAGCTG
CCCGCCGAGTCGTCGTGCTGGAACACGGTAAACCATGACCGAGAAGGAGATCGTGGACTATGTGGCCA
GCCAGGTTACAACCGCCAAGAAGCTGCGCGGTGGTGTGTTGTTTCGTGGACGAGGTGCCTAAAGGACTGA
CCGGCAAGTTGGACGCCCGCAAGATCCGCGAGATTCTCATTAAGGCCAAGAAGGGCGGCAAGATCGCCGT
GTAAATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTT
GGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAA]
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▪ **Nluc**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

Nluc: 88-597

Stop codon: 598-600

```
[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCGCCACCATGGTCTTCACACTCGAAGATTTTCGTTGGGGACTGGCGACAGACAGCCGGCTACAA  
CCTGGACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGTTCAGAATCTCGGGGTGTCCGTAACCTCCG  
ATCCAAAGGATTGTCTGAGCGGTGAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCGTATGAAGG  
TCTGAGCGGCGACCAAATGGGCCAGATCGAAAAAATTTTTAAGGTGGTGTACCCTGTGGATGATCATCACT  
TTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAACATGATCGACTATTTTCGGA  
CGGCCGTATGAAGGCATCGCCGTGTCGACGGCAAAAAGATCACTGTAACAGGGACCCTGTGGAACGGC  
AACAAAATTATCGACGAGCGCCTGATCAACCCCGACGGCTCCCTGCTGTTCCGAGTAACCATCAACGGAG  
TGACCGGCTGGCGGCTGTGCGAACGCATTCTGGCGTAAATCTAGACCTTCTGCGGGGCTTGCCCTTCTGGCC  
ATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAA  
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA]
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▪ **ARRB2-tevD-CaVT**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

ARRB2: 88-1311

3xGGGS flexible linker: 1312-1356

tevD: 1357-1419

MS2CP: 1420-1767

FCV-derived VPg: 1774-2106

Stop codon: 2107-2109

```
[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCGCCACCATGGGAGAAAAGCCTGGCACCAGAGTGTTCAAGAAGTCTAGCCCCAACTGCAAGC  
TGACCGTGTACCTCGGCAAGCGGGACTTCGTGGACCACCTGGACAAAGTGGATCCCGTGGATGGCGTGGT  
GCTGGTGGATCCTGACTACCTGAAGGACCGGAAGGTGTCGTGACCCTGACCTGCGCCTTCAGATACGGC  
AGAGAAGATCTGGACGTGCTGGGCCTGAGCTTCAGAAAGGACCTGTTTATCGCCACCTACCAGGCCTTTC  
CACCTGTGCCTAATCCTCCACGGCCTCTACCAGACTGCAGGACAGACTGCTGAGAAAGCTGGGCCAGCA  
CGCTCACCCCTTTTTCTTCACAATCCCTCAAAACCTGCCTTGCAGCGTGACACTGCAGCCCGGACCTGAGG  
ATACAGGCAAAGCTTGCGGCGTGGACTTCGAGATCAGAGCCTTCTGTGCCAAGAGCCTGGAAGAGAAGT  
CCCACAAGAGAAAACAGCGTGCGGCTGGTCATCAGAAAGGTGCAGTTCGCCCCTGAGAAGCCTGGACCTC  
AGCCTTCTGCCGAGACAACCAGACACTTCCTGATGAGCGACCCGAGCCTGCATCTGGAAGCCAGCCTCGA  
CAAAGAGCTGTACTACCACGGCGAGCCCTGAACGTCAACGTCCACGTGACCAACAACAGCACCAAGAC  
CGTGAAAAAGATCAAGGTGTCCGTGCGGCAGTACGCCGACATCTGCCTGTTTAGCACAGCCCAGTACAAG  
TGCCCCGTGGCTCAGCTGGAACAGGACGATCAAGTGTCCCCTAGCAGCACCTTCTGCAAGGTGTACACAA  
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GATACAAACCTGGCCAGCAGCACCATCGTGAAAGAGGGCGCCAACAAGAGGTGCTGGGCATCCTGGTG  
TCCTACAGAGTGAAAGTGAAGCTGGTGGTGTCCAGAGGCGGCGACGTTTCAGTGGAAGTGCCTTCTGTG  
TGATGCACCCCAAGCCTCACGATCACATCCCTCTGCCTAGACCTCAGAGCGCCGCTCCTGAAACAGATGTG
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CCCGTGGACACCAACCTGATCGAGTTCGACACCAACTACGCCACCGACGACGACATCGTGTTTCGAGGACT  
TCGCCCCGGCTGAGACTGAAGGGCATGAAGGACGACGACTACGACGATCAGCTTTGTGGCGGCGGAGGAT  
CTGGCGGAGGTGGAAGCGGAGGCGGTGGATCTGAGAACCTGTACTTCCAGTTCACAAAGAGCGGCGCCT  
GGAAGCTGCCTGTGTCTCTGGTTAAGGCTTCTAACTTTACTCAGTTCGTTCTCGTCGACAATGGCGGAACT  
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CACAGGCTTACAAAGTAACCTGTAGCGTTCGTCAGAGCTCTGCGCAGAATCGCAAATACACCATCAAAGT  
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AGAGGCGTGGCCCTTACAGACGACGAGTATGACGAATGGCGCGAGCACAACGCCACCAGAAAGCTGGAT  
CTGAGCGTGGAAGATTTCTGATGCTGCGGCACAGAGCCGCTCTGGGAGCTGATGATGCCGACGCCGTGA  
AGTTCAGATCCTGGTGGAACAGCAGAAGCCGGCTGGCCGACGATTACGAGGATGTGACCGTGATCGGCCAA  
AGGCGGCGTGAAGCACGAGAAGATCCGGACCAATACTCTGAGAGCCGTGGACAGAGGCTACGACGTGTC  
CTTCGCTGAAGAATGAATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCA  
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AA  
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA]

▪ **ARRB2-tevD-CaVT\_ver.2**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

ARRB2: 88-1311

3xGGGS flexible linker: 1312-1356

tevD: 1357-1419

2xGGGS flexible linker: 1420-1449

MS2CP: 1450-1797

FCV-derived VPg: 1804-2136

Stop codon: 2137-2139

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCgccaccatgGGAGAAAAGCCTGGCACCAGAGTGTTCAAGAAGTCTAGCCCCAACTGCAAGCTGA  
CCGTGTACCTCGGCAAGCGGGACTTCGTGGACCACCTGGACAAAGTGGATCCCGTGGATGGCGTGGTGCT  
GGTGGATCCTGACTACCTGAAGGACCGGAAGGTGTTTCGTGACCCTGACCTGCGCCTTCAGATACGGCAGA  
GAAGATCTGGACGTGCTGGGCCTGAGCTTCAGAAAGGACCTGTTTATCGCCACCTACCAGGCCTTTCCAC  
CTGTGCCTAATCCTCCACGGCCTCCTACCAGACTGCAGGACAGACTGCTGAGAAAGCTGGGCCAGCACGC  
TCACCCCTTTTTCTTCAATCCCTCAAACCTGCCTTGCAGCGTGACACTGCAGCCCGGACCTGAGGATA  
CAGGCAAAGCTTGCGGCGTGGACTTCGAGATCAGAGCCTTCTGTGCCAAGAGCCTGGAAGAGAAGTCCC  
ACAAGAGAAACAGCGTGCGGCTGGTCATCAGAAAGGTGCAGTTCGCCCTGAGAAGCCTGGACCTCAGC  
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AGAGCTGTACTACCACGGCGAGCCCTGAACGTCAACGTCCACGTGACCAACAACAGCACCAAGACCGT  
GAAAAGATCAAGGTGTCCGTGCGGCAGTACGCCGACATCTGCCTGTTTAGCACAGCCCAGTACAAGTGC

CCCGTGGCTCAGCTGGAACAGGACGATCAAGTGTCCCCTAGCAGCACCTTCTGCAAGGTGTACACAATCA  
CCCCTCTGCTGAGCGACAACAGAGAGAAGAGAGGACTGGCCCTGGACGGCAAGCTGAAGCACGAGGAT  
ACAAACCTGGCCAGCAGCACCATCGTGAAAGAGGGCGCCAACAAAGAGGTGCTGGGCATCCTGGTGTCC  
TACAGAGTGAAAGTGAAGCTGGTGGTGTCCAGAGGGCGGCGACGTTTCAGTGAACTGCCCTTCGTGCTG  
ATGCACCCCAAGCCTCACGATCACATCCCTCTGCCTAGACCTCAGAGCGCCGCTCCTGAAACAGATGTGCC  
CGTGGACACCAACCTGATCGAGTTCGACACCAACTACGCCACCGACGACGACATCGTGTTCGAGGACTTC  
GCCCCGGCTGAGACTGAAGGGCATGAAGGACGACGACTACGACGATCAGCTTTGTGGCGGCGGAGGATCT  
GGCGGAGGTGGAAGCGGAGGCGGTGGATCTGAGAACCTGTACTTCCAGTTCACAAAGAGCGGCGCCTGG  
AAGCTGCCTGTGTCTCTGGTTAAGGGCGGCGGCGGCTCCGGCGGCGGCGGCTCCGCTTCTAACTTTACTC  
AGTTCGTTCTCGTCGACAATGGCGGAACTGGCGACGTGACTGTGCCCAAGCAACTTCGCTAACGGGGT  
CGCTGAATGGATCAGCTCTAACTCGCGATCACAGGCTTACAAAGTAACCTGTAGCGTTCGTCAGAGCTCTG  
CGCAGAATCGCAAATACACCATCAAAGTCGAGGTGCCTAAAGGCGCATGGAGGTCTTACTTAAATATGGAA  
CTAACCATTTCAATTTTCGCCACGAATTCGACTGCGAGCTTATTGTTAAGGCAATGCAAGGTCTCCTAAA  
AGATGGAAACCCGATTCCCTCGGCCATCGCGGCCAACTCCGGCATCTACGGATCCGCCAAGGGCAAGACC  
AAGAGCAAAGTGGGCCCTACAGAGGCAGAGGCGTGGCCCTTACAGACGACGAGTATGACGAATGGCGC  
GAGCACAACGCCACCAGAAAGCTGGATCTGAGCGTGAAGATTTCTGATGCTGCGGCACAGAGCCGCT  
CTGGGAGCTGATGATGCCGACGCCGTGAAGTTCAGATCCTGGTGAACAGCAGAAGCCGGCTGGCCGAC  
GATTACGAGGATGTGACCGTGATCGGCAAAGGCGGCGTGAAGCACGAGAAGATCCGGACCAATACTCTGA  
GAGCCGTGGACAGAGGCTACGACGTGTCCTTCGCTGAAGAATGAATCTAGACCTTCTGCGGGGCTTGCCT  
TCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAA  
AAA  
AAA]

▪ **EGFP**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

EGFP: 88-801

Stop codon: 802-804

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGGCCATCCTGGTTCGAGCT  
GGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA  
GCTGACCCTGAAGTTCATCTGCACCACCGCAAGCTGCCCGTGCCCTGGCCACCCCTCGTGACCACCCTG  
ACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCA  
TGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCG  
AGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACG  
GCAACATCCTGGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCA  
GAAGAACGGCATCAAGGTGAAGTTCAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGA  
CCACTACCAGCAGAACACCCCATCGGCGACGGCCCGTGCTGCTGCCGACAACCACTACCTGAGCACC  
CAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCC  
GCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAATCTAGACCTTCTGCGGGGCTTGCCTTCTGGC

CATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAA  
AA  
AA]

▪ **CaVT**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

MS2CP: 88-435

FCV-derived VPg: 442-774

Stop codon: 775-777

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCGCCACCatgGCTTCTAACTTTACTCAGTTTCGTTCTCGTCGACAATGGCGGAACTGGCGACGTGA  
CTGTCGCCCAAGCAACTTCGCTAACGGGGTTCGCTGAATGGATCAGCTCTAACTCGCGATCACAGGCTTAC  
AAAGTAACCTGTAGCGTTTCGTCAGAGCTCTGCGCAGAATCGCAAATACACCATCAAAGTCGAGGTGCCTA  
AAGGCGCATGGAGGTCTTACTTAAATATGGAACCTAACCATTTCCAAATTTTCGCCACGAATTCGACTGCGAG  
CTTATTGTTAAGGCAATGCAAGGTCTCTAAAAGATGGAAACCCGATTCCCTCGGCCATCGCGGCCAACTC  
CGGCATCTACGGATCCGCCAAGGGCAAGACCAAGAGCAAAGTGGGCCCTACAGAGGCAGAGGCGTGGC  
CCTTACAGACGACGAGTATGACGAATGGCGCGAGCACAACGCCACCAGAAAGCTGGATCTGAGCGTGGA  
AGATTCCTGATGCTGCGGCACAGAGCCGCTCTGGGAGCTGATGATGCCGACGCCGTGAAGTTCAGATCC  
TGGTGGAACAGCAGAAGCCGGCTGGCCGACGATTACGAGGATGTGACCGTGATCGGCAAAGGCGGCGTG  
AAGCACGAGAAGATCCGGACCAATACTCTGAGAGCCGTGGACAGAGGCTACGACGTGTCTTCGCTGAA  
GAATGAATCTAGACCTTCTGCGGGGCTTGCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTC  
TTGGTCTTTGAATAAAGCCTGAGTAGGAAA  
AA  
AAAAAAAAAAAAAAAA]

▪ **TEVp**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

TEV protease (S219V mutant): 88-792

Stop codon: 793-795

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCgccaccatgGAGAGCCTGTTAAGGGCCCCAGAGACTACAACCCCATCAGCTCCACCATCTGCCA  
CCTGACCAATGAGTCCGATGGCCACACCACAAGCCTGTACGGCATCGGCTTCGGCCCCTTCATCATACCA  
ACAAGCACCTGTTCAGACGGAACAACGGCACCCCTGCTGGTGCAGTCTCTGCACGGCGTGTTCAAAGTGA  
AGAATACCACCACACTGCAGCAGCACCTGATCGACGGCCGGGACATGATCATCATCAGAATGCCAAGGA  
CTTCCCGCCTTTTCCACAGAAGCTGAAGTTCAGAGAGCCCCAGCGCGAGGAAAGAATCTGCCTGGTCAAC  
ACCAACTTCCAGACCAAGAGCATGTCCTCCATGGTGTCCGATACCAGCTGCACATTCCTTAGCAGCGACG  
GCATCTTCTGGAAGCACTGGATTACAGACCAAGGACGGCCAGTGTGGCAGCCCTCTGGTGTCTACAAGAGA  
TGGCTTCATCGTGGGCATCCACAGCGCCAGCAACTTCACCAATACCAACAACCTACTTCACCAGCGTGCCG



AAGAACTTCATGGAAGCTGCTGACCAATCAAGAGGCTCAGCAGTGGGTTTCCGGCTGGCGGCTGAATGCTG  
ATTCTGTGCTGTGGGGCGGACACAAGGTGTTTCATGGTCAAGCCCAGGAACCCCTCCAGCCTGTGAAAGA  
GGCCACACAGCTGATGAACTGAATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTC  
CCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
AA  
AA

▪ **TEVp (C151A)**

T7 promoter (for CleanCap AG Reagent): 11-30  
Kozak sequence (including start codon): 79-87  
TEV protease (C151A/S219V mutant): 88-792  
Stop codon: 793-795

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCGCCACCcatgGAGAGCCTGTTAAGGGCCCCAGAGACTACAACCCCATCAGCTCCACCATCTGC  
CACCTGACCAATGAGTCCGATGGCCACACCACAAGCCTGTACGGCATCGGCTTCGGCCCCTTCATCATCAC  
CAACAAGCACCTGTTTCAGACGGAACAACGGCACCCCTGCTGGTGCAGTCTCTGCACGGCGTGTTCAAAGT  
GAAGAATACCACCACACTGCAGCAGCACCTGATCGACGGCCGGGACATGATCATCATCAGAATGCCCAAG  
GACTTCCCGCCTTTTCCACAGAAGCTGAAGTTCAGAGAGCCCCAGCGGAGGAAAGAATCTGCCTGGTCA  
CCACCAACTTCCAGACCAAGAGCATGTCTCCATGGTGTCCGATACCAGCTGCACATTCCCTAGCAGCGAC  
GGCATCTTCTGGAAGCACTGGATTCAGACCAAGGACGGCCAGGCCGAGCCCTCTGGTGTCTACAAGAG  
ATGGCTTCATCGTGGGCATCCACAGCGCCAGCAACTTCACCAATACCAACAACACTTCCAGCGTGCCG  
AAGAACTTCATGGAAGCTGCTGACCAATCAAGAGGCTCAGCAGTGGGTTTCCGGCTGGCGGCTGAATGCTG  
ATTCTGTGCTGTGGGGCGGACACAAGGTGTTTCATGGTCAAGCCCAGGAACCCCTCCAGCCTGTGAAAGA  
GGCCACACAGCTGATGAACTGAATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTC  
CCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
AA  
AA]

▪ **AVPR2-TEVp**

T7 promoter (for CleanCap AG Reagent): 11-30  
Kozak sequence (including start codon): 79-87  
Human AVPR2: 88-1197  
3xGGGS flexible linker: 1198-1242  
TEV protease (S219V mutant): 1243-1947  
Stop codon: 1948-1950

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCGCCACCATGCTGATGGCCTCTACAACATCTGCCGTGCCTGGACACCCTAGCTGCCTTCTCTG  
CCTAGCAACAGCAGCCAAGAGAGGGCCCCTGGATACCAGAGATCCTCTGCTGGCCAGAGCCGAAGTGGCC  
CTGCTGTCTATCGTGTGTTGTGGCCGTGGCTCTGTCCAACGGACTGGTTCCTTGTGCCCTGGCTCGGAGAGG  
AAGAAGAGGACATTGGGCCCTATCCACGTGTTTCATCGGCCATCTGTGTCTGGCCGATCTGGCTGTGGCAC

TGTTTCAGGTTCTGCCTCAGCTGGCCTGGAAGGCCACCGATAGATTTCAGAGGCCCGACGCTCTGTGCAG  
AGCCGTGAAATACCTGCAGATGGTCGGGATGTACGCCAGCAGCTACATGATCCTGGCCATGACACTGGACC  
GGCACAGAGCCATCTGTAGACCCATGCTGGCCTACAGACATGGCTCTGGCGCCCACTGGAATAGACCTGT  
GCTTGTGGCCTGGGCCTTCAGCCTGCTTCTGTCTCTGCCCCAGCTGTTTCATCTTCGCCCAGAGAAATGTGG  
AAGGCGGCAGCGGCATTACAGATTGCTGGCCTGTTTTGCCGAGCCTTGGGGTAGAAGAACCTACGTGAC  
ATGGATCGCCCTGATGGTGTTCGTGGCCCTACACTGGGAATTGCCGCTTGCCAGGTGCTGATCTTCAGAG  
AGATCCACGCCAGCCTGGTGCCAGGACCTTCTGAAAGACCTGGCGGACGCAGACGGGGAAGAAGAACA  
GGATCTCCTGGCGAAGGCGCCCATGTGTCTGCTGCCGTGGCCAAGACAGTGCCGATGACACTCGTGATCG  
TGGTGGTGTACGTGCTGTGCTGGGCCCCATTCTTTCTGGTGCAACTGTGGGCCCGCCTGGGATCCTGAAGCT  
CCTCTTGAAGGCGCTCCCTTCGTGCTGCTGATGCTGCTGGCTAGCCTGAACTCCTGCACAAACCCCTGGAT  
CTACGCCTCCTTCAGCAGCAGCGTGTCCAGCGAGCTGAGAAGCCTGCTGTGTTGTGCCAGAGGCAGGAC  
ACCTCCATCTCTGGGACCTCAGGATGAGAGCTGTACCACCGCCTCTTCTAGCCTGGCCAAGGATACAAGTT  
CTGGCGGCGGAGGATCTGGCGGAGGTGGAAGCGGAGGCGGCGGATCTGAGAGCCTGTTTAAGGGCCCCA  
GAGACTACAACCCCATCAGCTCCACCATCTGCCACCTGACCAATGAGTCCGATGGCCACACCACAAGCCT  
GTACGGCATCGGCTTCGGCCCCCTTCATCATCACCAACAAGCACCTGTTTCAGACGGAACAACGGCACCCCTG  
CTGGTGCAGTCTCTGCACGGCGTGTCAAAGTGAAGAATACCACCACACTGCAGCAGCACCTGATCGACG  
GCCGGGACATGATCATCATCAGAATGCCCAAGGACTTCCCGCCTTTCCACAGAAGCTGAAGTTCAGAGA  
GCCCCAGCGCGAGGAAAGAATCTGCCTGGTCAACCACCAACTTCCAGACCAAGAGCATGTCCTCCATGGTG  
TCCGATACCAGCTGCACATTCCCTAGCAGCGACGGCATCTTCTGGAAGCACTGGATTTCAGACCAAGGACG  
GCCAGTGTGGCAGCCCTCTGGTGTCTACAAGAGATGGCTTCATCGTGGGCATCCACAGCGCCAGCAACTT  
CACCAATACCAACAACACTTTCACCAGCGTGCCGAAGAACTTCATGGAAGTCTGACCAATCAAGAGGCT  
CAGCAGTGGGTTTCCGGCTGGCGGCTGAATGCTGATTCTGTGCTGTGGGGCGGACACAAGGTGTTTCATGG  
TCAAGCCCGAGGAACCCCTCCAGCCTGTGAAAGAGGCCACACAGCTGATGAACTGAATCTAGACCTTCTG  
CGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCT  
GAGTAGGAA  
AA]

▪ **AVPR2-TEVpΔ**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

Human AVPR2: 88-1197

3xGGGS flexible linker: 1198-1242

Carboxy-terminally truncated TEVp (S219V mutant): 1243-1896

Stop codon: 1897-1899

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCgccaccATGCTGATGGCCTCTACAACATCTGCCGTGCCTGGACACCCTAGCCTGCCTTCTCTGCC  
TAGCAACAGCAGCCAAGAGAGGGCCCTGGATAACCAGAGATCCTCTGCTGGCCAGAGCCGAACTGGCCCT  
GCTGTCTATCGTGTGTTGTGGCCGTGGCTCTGTCCAACGGACTGGTTCTTGCTGCCCTGGCTCGGAGAGGAA  
GAAGAGGACATTGGGCCCTATCCACGTGTTTCATCGGCCATCTGTGTCTGGCCGATCTGGCTGTGGCACTG  
TTTCAGGTTCTGCCTCAGCTGGCCTGGAAGGCCACCGATAGATTTCAGAGGCCCGACGCTCTGTGCAGAG

CCGTGAAATACCTGCAGATGGTCGGGATGTACGCCAGCAGCTACATGATCCTGGCCATGACACTGGACCGG  
CACAGAGCCATCTGTAGACCCATGCTGGCCTACAGACATGGCTCTGGCGCCACTGGAATAGACCTGTGCT  
TGTGGCCTGGGCCTTCAGCCTGCTTCTGTCTCTGCCCCAGCTGTTTCATCTTCGCCAGAGAAATGTGGAAG  
GCGGCAGCGGCGTTACAGATTGCTGGGCTGTTTTGCCGAGCCTTGGGGTAGAAGAACCTACGTGACATG  
GATCGCCCTGATGGTGTTCGTGGCCCCTACACTGGGAATTGCCGCTTGCCAGGTGCTGATCTTCAGAGAGA  
TCCACGCCAGCCTGGTGCCAGGACCTTCTGAAAGACCTGGCGGACGCAGACGGGGAAGAAGAACAGGAT  
CTCCTGGCGAAGGCGCCCATGTGTCTGCTGCCGTGGCCAAGACAGTGC GGATGACACTCGTGATCGTGGT  
GGTGTACGTGCTGTGCTGGGCCCCATTCTTTCTGGTGCAACTGTGGGCCGCCTGGGATCCTGAAGCTCCTC  
TTGAAGGCGCTCCCTTCGTGCTGCTGATGCTGCTGGCTAGCCTGAACTCCTGCACAAACCCCTGGATCTAC  
GCCTCCTTCAGCAGCAGCGTGTCCAGCGAGCTGAGAAGCCTGCTGTGTTGTGCCAGAGGCAGGACACCT  
CCATCTCTGGGACCTCAGGATGAGAGCTGTACCACCGCCTCTTCTAGCCTGGCCAAGGATACAAGTTCTGG  
CGGCGGAGGATCTGGCGGAGGTGGAAGCGGAGGCGGCGGATCTGAGAGCCTGTTTAAAGGGCCCCAGAGA  
CTACAACCCCATCAGCTCCACCATCTGCCACCTGACCAATGAGTCCGATGGCCACACCACAAGCCTGTACG  
GCATCGGCTTCGGCCCCCTTCATCATCAACAACAAGCACCTGTTTCAGACGGAACAACGGCACCCCTGCTGGT  
GCAGTCTCTGCACGGCGTGTTCAAAGTGAAGAATACCACCACACTGCAGCAGCACCTGATCGACGGCCGG  
GACATGATCATCATCAGAATGCCCAAGGACTTCCCGCCTTTTCCACAGAAGCTGAAGTTCAGAGAGCCCC  
AGCGCGAGGAAAGAATCTGCCTGGTCAACCAACTTCCAGACCAAGAGCATGTCCTCCATGGTGTCCGA  
TACCAGCTGCACATTCCCTAGCAGCGACGGCATCTTCTGGAAGCACTGGATTTCAGACCAAGGACGGCCAG  
TGTGGCAGCCCTCTGGTGTCTACAAGAGATGGCTTCATCGTGGGCATCCACAGCGCCAGCAACTTCACCA  
ATACCAACAATACTTCAACAGCGTGCCGAAGAATACTCATGGAACTGCTGACCAATCAAGAGGCTCAGCA  
GTGGGTTTCCGGCTGGCGGCTGAATGCTGATTCTGTGCTGTGGGGCGGACACAAGGTGTTTCATGGTCTGA  
ATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACACTGTACCTCTTGGTC  
TTTGAATAAAGCCTGAGTAGGAA  
AA  
AAAAAAAAA]

▪ **AVPR2-TEVp $\Delta$ T30A**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

Human AVPR2: 88-1197

3xGGGS flexible linker: 1198-1242

Carboxy-terminally truncated TEVp (T30A/S219V mutant): 1243-1896

Stop codon: 1897-1899

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTC<sub>gccacc</sub>ATGCTGATGGCCTCTACAACATCTGCCGTGCCTGGACACCCTAGCCTGCCTTCTCTGCC  
TAGCAACAGCAGCCAAGAGAGGCCCCCTGGATACCAGAGATCCTCTGCTGGCCAGAGCCGAACTGGCCCT  
GCTGTCTATCGTGTGTTGTGGCCGTGGCTCTGTCCAACGGACTGGTTCTTGCTGCCCTGGCTCGGAGAGGAA  
GAAGAGGACATTGGGCCCCATCCACGTGTTTCATCGGCCATCTGTGTCTGGCCGATCTGGCTGTGGCACTG  
TTTCAGGTTCTGCCTCAGCTGGCCTGGAAGGCCACCGATAGATTTCAGAGGCCCCGACGCTCTGTGCAGAG  
CCGTGAAATACCTGCAGATGGTCGGGATGTACGCCAGCAGCTACATGATCCTGGCCATGACACTGGACCGG

CACAGAGCCATCTGTAGACCCATGCTGGCCTACAGACATGGCTCTGGCGCCCACTGGAATAGACCTGTGCT  
TGTGGCCTGGGCCTTCAGCCTGCTTCTGTCTCTGCCCCAGCTGTTTCATCTTCGCCCAGAGAAATGTGGAAG  
GCGGCAGCGGCGTTACAGATTGCTGGGCCTGTTTTGCCGAGCCTTGGGGTAGAAGAACCTACGTGACATG  
GATCGCCCTGATGGTGTTCGTGGCCCCTACACTGGGAATTGCCGCTTGCCAGGTGCTGATCTTCAGAGAGA  
TCCACGCCAGCCTGGTGCCAGGACCTTCTGAAAGACCTGGCGGACGCAGACGGGGAAGAAGAACAGGAT  
CTCCTGGCGAAGGCGCCCATGTGTCTGCTGCCGTGGCCAAGACAGTGC GGATGACACTCGTGATCGTGGT  
GGTGTACGTGCTGTGCTGGGCCCCATTCTTTCTGGTGCAACTGTGGGCCGCCTGGGATCCTGAAGCTCCTC  
TTGAAGGCGCTCCCTTCGTGCTGCTGATGCTGCTGGCTAGCCTGAACTCCTGCACAAACCCCTGGATCTAC  
GCCTCCTTCAGCAGCAGCGTGTCCAGCGAGCTGAGAAGCCTGCTGTGTTGTGCCAGAGGCAGGACACCT  
CCATCTCTGGGACCTCAGGATGAGAGCTGTACCACCGCCTCTTCTAGCCTGGCCAAGGATACAAGTTCTGG  
CGGCGGAGGATCTGGCGGAGGTGGAAGCGGAGGCGGCGGATCTGAGAGCCTGTTTAAGGGCCCCAGAGA  
CTACAACCCCATCAGCTCCACCATCTGCCACCTGACCAATGAGTCCGATGGCCACACCGCCAGCCTGTACG  
GCATCGGCTTCGGCCCCCTTCATCATCACCAACAAGCACCTGTTTCAGACGGAACAACGGCACCCCTGCTGGT  
GCAGTCTCTGCACGGCGTGTTCAAAGTGAAGAATACCACCACACTGCAGCAGCACCTGATCGACGGCCGG  
GACATGATCATCATCAGAATGCCCAAGGACTTCCCGCCTTTTCCACAGAAGCTGAAGTTCAGAGAGCCCC  
AGCGCGAGGAAAGAATCTGCCTGGTCAACCACCAACTTCCAGACCAAGAGCATGTCTCCATGGTGTCCGA  
TACCAGCTGCACATTCCCTAGCAGCGACGGCATCTTCTGGAAGCACTGGATTTCAGACCAAGGACGGCCAG  
TGTGGCAGCCCTCTGGTGTCTACAAGAGATGGCTTCATCGTGGGCATCCACAGCGCCAGCAACTTCACCA  
ATACCAACAACACTTTCACCAGCGTGCCGAAGAACTTCATGGAAGTCTGACCAATCAAGAGGCTCAGCA  
GTGGGTTTCCGGCTGGCGGCTGAATGCTGATTCTGTGCTGTGGGGCGGACACAAGGTGTTTCATGGTCTGA  
ATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTGGTC  
TTTGAATAAAGCCTGAGTAGGAA  
AA  
AAAAAAAAA]

▪ **AVPR2-TEVpΔS135N**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

Human AVPR2: 88-1197

3xGGGS flexible linker: 1198-1242

Carboxy-terminally truncated TEVp (S135N/S219V mutant): 1243-1896

Stop codon: 1897-1899

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCgccaccATGCTGATGGCCTCTACAACATCTGCCGTGCCTGGACACCCTAGCCTGCCTTCTCTGCC  
TAGCAACAGCAGCCAAGAGAGGGCCCCTGGATAACCAGAGATCCTCTGCTGGCCAGAGCCGAACTGGCCCT  
GCTGTCTATCGTGTGTTGTGGCCGTGGCTCTGTCCAACGGACTGGTTCTTGCTGCCCTGGCTCGGAGAGGAA  
GAAGAGGACATTGGGCCCCTATCCACGTGTTTCATCGGCCATCTGTGTCTGGCCGATCTGGCTGTGGCACTG  
TTTCAGGTTCTGCCTCAGCTGGCCTGGAAGGCCACCGATAGATTTCAGAGGCCCCGACGCTCTGTGCAGAG  
CCGTGAAATACCTGCAGATGGTCGGGATGTACGCCAGCAGCTACATGATCCTGGCCATGACACTGGACCGG  
CACAGAGCCATCTGTAGACCCATGCTGGCCTACAGACATGGCTCTGGCGCCCACTGGAATAGACCTGTGCT

TGTGGCCTGGGCCTTCAGCCTGCTTCTGTCTCTGCCCCAGCTGTTTCATCTTCGCCAGAGAAATGTGGAAG  
GCGGCAGCGGCGTTACAGATTGCTGGGCCTGTTTTGCCGAGCCTTGGGGTAGAAGAACCTACGTGACATG  
GATCGCCCTGATGGTGTTCGTGGCCCCTACACTGGGAATTGCCGCTTGCCAGGTGCTGATCTTCAGAGAGA  
TCCACGCCAGCCTGGTGCCAGGACCTTCTGAAAGACCTGGCGGACGCAGACGGGGAAGAAGAACAGGAT  
CTCCTGGCGAAGGCGCCCATGTGTCTGCTGCCGTGGCCAAGACAGTGCGGATGACACTCGTGATCGTGGT  
GGTGTACGTGCTGTGCTGGGCCCCATTCTTTCTGGTGCAACTGTGGGCGCCTGGGATCCTGAAGCTCCTC  
TTGAAGGCGCTCCCTTCGTGCTGCTGATGCTGCTGGCTAGCCTGAACTCCTGCACAAACCCCTGGATCTAC  
GCCTCCTTCAGCAGCAGCGTGTCCAGCGAGCTGAGAAGCCTGCTGTGTTGTGCCAGAGGCAGGACACCT  
CCATCTCTGGGACCTCAGGATGAGAGCTGTACCACCGCCTCTTCTAGCCTGGCCAAGGATACAAGTTCTGG  
CGGCGGAGGATCTGGCGGAGGTGGAAGCGGAGGCGGCGGATCTGAGAGCCTGTTTAAAGGGCCCCAGAGA  
CTACAACCCCATCAGCTCCACCATCTGCCACCTGACCAATGAGTCCGATGGCCACACCACAAGCCTGTACG  
GCATCGGCTTCGGCCCCCTTCATCATACCAACAAGCACCTGTTTCAGACGGAACAACGGCACCCCTGCTGGT  
GCAGTCTCTGCACGGCGTGTCAAAGTGAAGAATACCACCACACTGCAGCAGCACCTGATCGACGGCCGG  
GACATGATCATCATCAGAATGCCCAAGGACTTCCCGCCTTTTCCACAGAAGCTGAAGTTCAGAGAGCCCC  
AGCGCGAGGAAAGAATCTGCCTGGTCAACCAACTTCCAGACCAAGAGCATGTCTCCATGGTGTCCGA  
TACCAGCTGCACATTCCCTAGCAGCGACGGCATCTTCTGGAAGCACTGGATTTCAGACCAAGGACGGCCAG  
TGTGGCAACCCTCTGGTGTCTACAAGAGATGGCTTCATCGTGGGCATCCACAGCGCCAGCAACTTCACCA  
ATACCAACAACACTTCCACAGCGTGCCGAAGAACTTCATGGAACTGCTGACCAATCAAGAGGCTCAGCA  
GTGGGTTTCCGGCTGGCGGCTGAATGCTGATTCTGTGCTGTGGGGCGGACACAAGGTGTTTCATGGTCTGA  
ATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTGGTC  
TTTGAATAAAGCCTGAGTAGGAA  
AA  
AAAAAAAAA]

▪ **EP3-TEVp $\Delta$ T30A**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

Human EP3 isoform I: 88-1254

A343-S371 of human AVPR2: 1255-1341

3xGGGS flexible linker: 1342-1386

Carboxy-terminally truncated TEVp (T30A/S219V mutant): 1387-2040

Stop codon: 2041-2043

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCgccaccATGAAGGAGACCCGGGGCTACGGCGGCGATGCCCCCTTCTGCACCAGGCTGAACCAC  
AGCTACACCGGCATGTGGGCCCCCGAGCGGAGTGCTGAGGCCAGGGGAAACCTGACCCGGCCTCCTGGC  
AGCGGAGAGGACTGCGGCAGCGTGAGTGTGGCTTTCCCTATCACCATGCTGCTGACCGGCTTCGTGGGCA  
ACGCTCTTGCCATGCTGCTGGTGAGCCGGAGCTACCGGCGGAGGGAGAGCAAGCGGAAGAAGAGCTTCC  
TGCTGTGCATCGGCTGGCTGGCCCTGACCGACCTGGTGGGCCAGCTGCTGACCACCCCGTGGTGATCGT  
GGTGTACCTGAGCAAGCAGCGGTGGGAGCACATCGACCCAGCGGCCGGCTGTGCACCTTCTTCGGCCTG  
ACCATGACCGTGTTTCGGCCTGAGCAGCCTGTTTCATCGCCAGCGCCATGGCCGTGGAGAGGGCCCTTGCCA

TCAGGGCTCCCCACTGGTACGCCAGCCACATGAAGACCCGGGCCACCCGGGCTGTTCTGCTGGGAGTGTG  
GCTTGCCGTGCTGGCCTTCGCTCTGCTGCCTGTGCTGGGCGTGGGCCAGTACACCGTGCAATGGCCTGGC  
ACATGGTGCTTCATCAGCACCGGCAGGGGAGGCAACGGCACCCAGCAGCAGCCACAACCTGGGGCAACCTG  
TTCTTCGCCAGTGCCTTCGCCTTCCTGGGCTGCTGGCCCTGACCGTGACCTTCAGCTGCAACCTGGCCAC  
CATCAAGGCCCTGGTGAGCCGGTGCCGGGCCAAGGCCACCGCTAGTCAGAGCAGCGCCAGTGGGGACG  
GATCACCACAGAGACCGCCATCCAGCTGATGGGCATCATGTGCGTGCTGAGCGTGTGCTGGAGCCCCCTG  
CTGATCATGATGCTGAAGATGATCTTCAACCAGACCAGCGTGGAGCACTGCAAGACCCACACCGAGAAGC  
AGAAGGAGTGCAACTTCTTCCTGATCGCCGTGCGGCTGGCCAGCCTGAACCAGATCCTGGACCCCTGGGT  
GTACCTGCTGCTGCGGAAGATCCTGCTGCGGAAGTTCTGCCAGATCCGGTACCACACCAACAACACTACGCC  
AGCAGCAGCACCCAGCCTGCCCTGCCAGTGCAGCAGCACCCCTGATGTGGAGCGACCACCTGGAGCGGGCC  
AGAGGCAGGACACCTCCATCTCTGGGACCTCAGGATGAGAGCTGTACCACCGCCTTTCTAGCCTGGCCA  
AGGATACAAGTTCTGGCGGCGGAGGATCTGGCGGAGGTGGAAGCGGAGGCGGCGGATCTGAGAGCCTGT  
TTAAGGGCCCCAGAGACTACAACCCCATCAGCTCCACCATCTGCCACCTGACCAATGAGTCCGATGGCCA  
CACCGCCAGCCTGTACGGCATCGGCTTCGGCCCCCTTCATCATACCAACAAGCACCTGTTTCAGACGGAAC  
AACGGCACCCCTGCTGGTGCAGTCTCTGCACGGCGTGTTCAAAGTGAAGAATACCACCACACTGCAGCAGC  
ACCTGATCGACGGCCGGGACATGATCATCATCAGAATGCCCAAGGACTTCCCGCCTTTTCCACAGAAGCTG  
AAGTTCAGAGAGCCCCAGCGGAGGAAAGAATCTGCCTGGTCACCACCAACTTCCAGACCAAGAGCATG  
TCCTCCATGGTGTCCGATACCAGCTGCACATTCCCTAGCAGCGACGGCATCTTCTGGAAGCACTGGATTCA  
GACCAAGGACGGCCAGTGTGGCAGCCCTCTGGTGTCTACAAGAGATGGCTTCATCGTGGGCATCCACAGC  
GCCAGCAACTTCACCAATAACCAACAACACTACTTCACCAGCGTGCCGAAGAACTTCATGGAAGTGTGACCA  
ATCAAGAGGCTCAGCAGTGGGTTTCCGGCTGGCGGCTGAATGCTGATTCTGTGCTGTGGGGCGGACACAA  
GGTGTTCATGGTCTGAATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCA  
CCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  
AA  
AA]

▪ **EP4-TEVp $\Delta$ T30A**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

Human EP4: 88-1548

A343-S371 of AVPR2: 1549-1635

3xGGGS flexible linker: 1636-1680

Carboxy-terminally truncated TEVp (T30A/S219V mutant): 1681-2334

Stop codon: 2335-2337

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTCgccaccATGAGCACCCCCGGCGTGAACAGCAGCGCCAGCCTGAGCCCCGACAGGCTGAACAGC  
CCCGTGACCATCCCCGCCGTGATGTTTCATCTTCGGCGTGGTGGGCAACCTGGTGGCCATCGTGGTGTGCTGTG  
CAAGAGCCGGAAGGAGCAGAAGGAGACCACCTTCTACACCCTGGTGTGCGGCCTGGCCGTGACCGACCT  
GCTGGGCACCCTGCTGGTGTGAGCCCCGTGACCATCGCTACCTACATGAAGGGCCAGTGGCCCCGGCGGACAG  
CCTCTGTGCGAGTACAGCACCTTCATCCTGCTGTTCTTCAGCCTGAGCGGCCTGAGCATCATCTGCGCCAT

GAGCGTGGAGCGGTACCTGGCCATCAACCACGCCTACTTCTACAGCCACTACGTGGACAAGCGGCTGGCC  
GGCCTGACCCTGTTTCGCCGTGTACGCCAGCAACGTGCTGTTCTGCGCCCTGCCCAACATGGGCCTGGGCA  
GCAGCCGGCTGCAGTACCCTGACACCTGGTGTTCATCGACTGGACCACCAACGTGACCGCCCACGCCGC  
CTACAGCTACATGTACGCCGGCTTCAGCAGCTTCCTGATCCTGGCCACCGTGTGTGCAACGTGCTGGTGT  
GCGGCGCCCTGCTGCGGATGCACCGGCAGTTCATGCGGCGGACCAGCCTGGGCACCGAGCAACACCATG  
CCGCTGCTGCTGCCAGCGTTGCTAGTCGGGGACACCCTGCTGCCAGTCCCGCCCTGCCTAGGCTGAGCGA  
TTTTCGGAGGCGGAGGAGCTTCAGGAGGATTGCCGGAGCCGAGATCCAGATGGTGATCCTGCTGATCGCC  
ACCAGCCTGGTGGTGTGCTGATCTGCAGCATCCCCCTGGTGGTGCGGGTGTTTCGTGAACCAGCTGTACCAGC  
CCAGCCTGGAGCGGGAGGTGAGCAAGAACCCCGACCTGCAGGCCATCCGGATCGCCAGCGTGAACCCCA  
TCCTGGACCCCTGGATCTACATCCTGCTGCGGAAGACCGTGTGAGCAAGGCCATCGAGAAGATCAAGTG  
CCTGTTCTGCCGGATCGGCGGCAGCCGGAGGGAGAGGAGCGGCCAACACTGCAGCGACAGCCAGCGGAC  
CAGCAGCGCCATGAGCGGCCACAGCAGGAGTTTCATCAGCCGGGAGCTGAAGGAGATCAGCAGCACCAG  
CCAGACCCTGCTGCCCCGACCTGAGCCTGCCCCGACCTGAGCGAGAACGGCCTGGGAGGAAGGAACCTGCT  
TCCCCGAGTGCCCCGCATGGGCCTGGCTCAGGAGGACACCACCAGCCTGCGGACCCTGCGGATCAGCGA  
GACCAGCGACAGCAGCCAGGGCCAAGATAGTGAGAGCGTGCTGCTGGTTGATGAGGCTGGAGGAAGCGG  
CAGGGCCCGACCTGCTCCCAAGGGCAGCAGCCTGCAGGTGACCTTCCCCAGCGAGACCCTGAACCTGAG  
CGAGAAGTGCATCGCCAGAGGCAGGACACCTCCATCTCTGGGACCTCAGGATGAGAGCTGTACCACCGCC  
TCTTCTAGCCTGGCCAAGGATAACAAGTTCTGGCGGCGGAGGATCTGGCGGAGGTGGAAGCGGAGGCGGC  
GGATCTGAGAGCCTGTTAAGGGCCCCAGAGACTACAACCCCATCAGCTCCACCATCTGCCACCTGACCA  
ATGAGTCCGATGGCCACACCGCCAGCCTGTACGGCATCGGCTTCGGCCCCTTCATCATCACCACAAGCAC  
CTGTTTCAGACGGAACAACGGCACCCCTGCTGGTGCAGTCTCTGCACGGCGTGTTCAAAGTGAAGAATACCA  
CCACACTGCAGCAGCACCTGATCGACGGCCGGACATGATCATCATCAGAATGCCCAAGGACTTCCCGCC  
TTTTCCACAGAAGCTGAAGTTCAGAGAGCCCCAGCGCGAGGAAAGAATCTGCCTGGTCACCACCAACTTC  
CAGACCAAGAGCATGTCCTCCATGGTGTCCGATAACCAGCTGCACATTCCCTAGCAGCGACGGCATCTTCTG  
GAAGCACTGGATTCAGACCAAGGACGGCCAGTGTGGCAGCCCTCTGGTGTCTACAAGAGATGGCTTCATC  
GTGGGCATCCACAGCGCCAGCAACTTCACCAATACCAACAACACTTACCAGCGTGCCGAAGAACTTCA  
TGAACTGCTGACCAATCAAGAGGCTCAGCAGTGGGTTTCCGGCTGGCGGCTGAATGCTGATTCTGTGCT  
GTGGGGCGGACACAAGGTGTTTCATGGTCTGAATCTAGACCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCT  
TCTTCTCTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAA  
AA  
AA]

▪ **B2-TEVp $\Delta$ T30A**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

Human B2: 88-1257

A343-S371 of human AVPR2: 1258-1344

3xGGGS flexible linker: 1345-1389

Carboxy-terminally truncated TEVp (T30A/S219V mutant): 1390-2043

Stop codon: 2044-2046

[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA  
CACCGGTC<sup>gccacc</sup>ATGTTTAGCCCTTGGAAGATCAGCATGTTCTGAGCGTGCGGAGGATAGCGTGCCAAC  
CACAGCCAGCTTTAGCGCCGACATGCTGAACGTGACACTGCAGGGCCCTACACTGAACGGCACATTTCGCC  
CAGAGCAAGTGCCCTCAGGTGGAATGGCTCGGCTGGCTGAATACGATCCAGCCTCCTTTCCTGTGGGTGC  
TGTTTCGTGCTGGCCACACTGGAAAACATCTTCGTGCTGAGCGTGTTCTGCCTGCACAAGAGCAGCTGTAC  
CGTGGCCGAGATCTACCTGGGAAATCTGGCCGCTGCCGATCTGATCCTGGCTTGCGGACTTCCTTTCTGGG  
CCATCACCATCAGCAACAACCTTCGACTGGCTGTTCCGGCGAGACACTGTGCAGAGTGGTCAACGCCATCAT  
CAGCATGAACCTGTACAGCAGCATCTGCTTTCTGATGCTGGTGTCCATCGACCCGGTATCTGGCCCTGGTCA  
AGACCATGAGCATGGGCAGAATGCGGGGCGTCAGATGGGCCAAGCTGTACTCTCTGGTCACTCTGGGGCTG  
TACCCTGCTGCTGTCTAGCCCCATGCTCGTGTTCGGGACCATGAAGGAATACTCCGACGAGGGGCCACAACG  
TGACCGCCTGTGTGATCAGCTACCCAGCCTGATCTGGGAAGTGTTACCAACATGCTGCTGAATGTCGTG  
GGCTTCCTGCTGCCTCTGAGCGTGATCACCTTCTGCACCATGCAGATCATGCAGGTCCTGCGGAACAACGA  
GATGCAGAAGTTCAAAGAGATCCAGACCGAGCGGAGAGCCACCGTGCTGGTTCTGGTTGTGCTGCTCCTG  
TTCATCATCTGCTGGCTGCCCTTCCAGATCAGCACCTTCTGGACACCCTGCACAGACTGGGCATCCTGTG  
CAGCTGCCAGGACGAGAGAATCATCGATGTGATCACCCAGATCGCCAGCTTCATGGCCTACAGCAACAGC  
TGCCTGAATCCTCTGGTGTACGTGATCGTGGGCAAGCGCTTCAGAAAAGAAAAGCTGGGAAGTCTACCAGG  
GCGTGTGCCAGAAAGGCGGCTGTAGATCTGAGCCCATCCAGATGGAAAACCTCATGGGCACCCTGCGGAC  
CAGCATCTCCGTGGAAAGACAGATCCACAAGCTGCAGGATTGGGCCGGCTCTAGACAAGCCAGAGGCAG  
GACACCTCCATCTCTGGGACCTCAGGATGAGAGCTGTACCACCGCCTTCTAGCCTGGCCAAGGATACAA  
GTTCTGGCGGCGGAGGATCTGGCGGAGGTGGAAGCGGAGGCGGGCGGATCTGAGAGCCTGTTTAAGGGCC  
CCAGAGACTACAACCCCATCAGCTCCACCATCTGCCACCTGACCAATGAGTCCGATGGCCACACCGCCAG  
CCTGTACGGCATCGGCTTCGGCCCCTTCATCATCACCAACAAGCACCTGTTTCAGACGGAACAACGGCACC  
CTGCTGGTGCAGTCTCTGCACGGCGTGTTCAAAGTGAAGAATACCACCACACTGCAGCAGCACCTGATCG  
ACGGCCGGGACATGATCATCATCAGAATGCCAAGGACTTCCC GCCTTTTCCACAGAAGCTGAAGTTCAG  
AGAGCCCCAGCGCGAGGAAAGAATCTGCCTGGTCAACCACCAACTTCCAGACCAAGAGCATGTCCTCCATG  
GTGTCCGATAACCAGCTGCACATTCCCTAGCAGCGACGGCATCTTCTGGAAGCACTGGATTCAGACCAAGG  
ACGGCCAGTGTGGCAGCCCTCTGGTGTCTACAAGAGATGGCTTCATCGTGGGCATCCACAGCGCCAGCAA  
CTTCACCAATACCAACAACACTTTCACCAGCGTGCCGAAGA ACTTCATGGA ACTGCTGACCAATCAAGAG  
GCTCAGCAGTGGGTTTCCGGCTGGCGGCTGAATGCTGATTCTGTGCTGTGGGGCGGACACAAGGTGTTCA  
TGGTCTGAATCTAGACCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACC  
TCTTGGTCTTTGAATAAAGCCTGAGTAGGAA  
AA  
AAAAAAAAAAAAAAAAAAAA]

▪ **EP3V2-TEVp $\Delta$ T30A**

- T7 promoter (for CleanCap AG Reagent): 11-30
- Kozak sequence (including start codon): 79-87
- K2-R350 of human EP3 isoform I: 88-1134
- S327-S371 of human AVPR2: 1135-1269
- 3xGGGS flexible linker: 1270-1314



Carboxy-terminally truncated TEVp (T30A/S219V mutant): 1315-1968

Stop codon: 1969-1971

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[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA
CACCGGTCGCCACCATGAAGGAGACCCGGGGCTACGGCGGGGATGCCCCCTTCTGCACCAGGCTGAACC
ACAGCTACACCGGCATGTGGGCCCCCGAGCGGAGTGCTGAGGCCAGGGGAAACCTGACCCGGCCTCCTG
GCAGCGGAGAGGACTGCGGCAGCGTGAGTGTGGCTTTCCCTATCACCATGCTGCTGACCCGGCTTCGTGGG
CAACGCTCTTGCCATGCTGCTGGTGAGCCGGAGCTACCGGCGGAGGGAGAGCAAGCGGAAGAAGAGCTT
CCTGCTGTGCATCGGCTGGCTGGCCCTGACCGACCTGGTGGGCCAGCTGCTGACCACCCCGTGGTGATC
GTGGTGTACCTGAGCAAGCAGCGGTGGGAGCACATCGACCCAGCGGCCGGCTGTGCACCTTCTTCGGCC
TGACCATGACCGTGTTCGGCCTGAGCAGCCTGTTTCATCGCCAGCGCCATGGCCGTGGAGAGGGCCCTTGC
CATCAGGGCTCCCCACTGGTACGCCAGCCACATGAAGACCCGGGCCACCCGGGCTGTTCTGCTGGGAGTG
TGGCTTGCCGTGCTGGCCTTCGCTCTGCTGCCTGTGCTGGGCGTGGGCCAGTACACCGTGCAATGGCCTG
GCACATGGTGCTTCATCAGCACCCGGCAGGGGAGGCAACGGCACCAGCAGCAGCCACAACCTGGGGCAACC
TGTTCTTCGCCAGTGCCTTCGCTTCCTGGGCCTGCTGGCCCTGACCGTGACCTTCAGCTGCAACCTGGCC
ACCATCAAGGCCCTGGTGAGCCGGTGCCGGGCCAAGGCCACCGCTAGTCAGAGCAGCGCCAGTGGGGA
CGGATCACACAGAGACCGCCATCCAGCTGATGGGCATCATGTGCGTGCTGAGCGTGTGCTGGAGCCCC
TGCTGATCATGATGCTGAAGATGATCTTCAACCAGACCAGCGTGAGCACTGCAAGACCCACACCGAGAA
GCAGAAGGAGTGCAACTTCTTCCTGATCGCCGTGCGGCTGGCCAGCCTGAACCAGATCCTGGACCCCTGG
GTGTACCTGCTGCTGCGGTCTTCAGCAGCAGCGTGTCAGCGAGCTGAGAAGCCTGCTGTGTTGTGCCA
GAGGCAGGACACCTCCATCTCTGGGACCTCAGGATGAGAGCTGTACCACCGCCTCTTCTAGCCTGGCCAA
GGATAAAGTTCTGGCGGCGGAGGATCTGGCGGAGGTGGAAGCGGAGGGCGGCGGATCTGAGAGCCTGTT
TAAGGGCCCCAGAGACTACAACCCCATCAGCTCCACCATCTGCCACCTGACCAATGAGTCCGATGGCCAC
ACCGCCAGCCTGTACGGCATCGGCTTCGGCCCTTCATCATCACCAACAAGCACCTGTTTCAGACGGAACA
ACGGCACCCCTGCTGGTGCAGTCTCTGCACGGCGTGTTCAAAAGTGAAGAATACCACCACACTGCAGCAGCA
CCTGATCGACGGCCGGGACATGATCATCATCAGAATGCCCAAGGACTTCCCGCCTTTTCCACAGAAGCTGA
AGTTCAGAGAGCCCCAGCGCGAGGAAAGAATCTGCCTGGTCACCACCAACTTCCAGACCAAGAGCATGT
CCTCCATGGTGTCCGATAACCAGCTGCACATTCCTAGCAGCGACGGCATCTTCTGGAAGCACTGGATTAG
ACCAAGGACGGCCAGTGTGGCAGCCCTCTGGTGTCTACAAGAGATGGCTTCATCGTGGGCATCCACAGCG
CCAGCAACTTCACCAATACCAACAACACTTTCACCAGCGTGCCGAAGAACTTCATGGAACCTGCTGACCAA
TCAAGAGGCTCAGCAGTGGGTTTCCGGCTGGCGGCTGAATGCTGATTCTGTGCTGTGGGGCGGACACAAG
GTGTTTCATGGTCTGAATCTAGACCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTCCTTGCAC
CTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA]
```

▪ **2xScMS2(C)-IL1Ra-HiBiT**

T7 promoter (for CleanCap AG Reagent): 11-30

Stabilized 2xMS2-binding motif (C-variant): 51-117

Kozak sequence (including start codon): 125-133

Human IL-1Ra isoform II: 134-670

2xGSSG linker: 671-694

HiBiT: 695-727

Stop codon: 728-730

```
[CAGTGAATTGTAATACGACTCACTATAAGGTCAGATCCGCTAGCGGATCCgggagcAggtgAGGATCACCCATc
TgccacgagcgAggtgAGGATCACCCATcTcgctcgtgttcccACCGGTCgccaccATGGCTCTGGCCGACCTGTATGAAGAA
GGTGGCGGCGGAGGCGGAGAGGGGCGAAGATAATGCCGACAGCAAAGAGACAATCTGCAGACCCAGCGG
CCGGAAGTCCTCTAAGATGCAGGCCTTCAGAATCTGGGACGTGAACCAGAAAACCTTCTACCTGCGGAAC
AATCAGCTGGTGGCCGGCTATCTGCAGGGCCCCAATGTGAACCTGGAAGAGAAGATCGACGTGGTGCCCA
TCGAGCCCCACGCTCTGTTTCTTGGAATCCACGGCGGCAAGATGTGCCTGAGCTGTGTGAAGTCTGGCGA
CGAGACACGGCTGCAGCTGGAAGCCGTGAACATCACCGACCTGAGCGAGAACCGGAAGCAGGACAAGA
GATTCGCCTTCATCAGAAGCGACAGCGGCCCCACCACAAGCTTTGAGTCTGCTGCTTGCCCTGGCTGGTTc
CTGTGTACAGCCATGGAAGCCGACCAGCCTGTGTCTCTGACCAACATGCCTGACGAGGGCGTGATGGTCA
CCAAGTTCTACTTCCAAGAGGACGAGGGCAGCAGCGGCGGCAGCAGCGGCGTGAGCGGCTGGCGGCTGT
TCAAGAAGATTAGCTGAATCTAGACCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGC
ACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA]
```

▪ **IL1Ra-HiBiT**

T7 promoter (for CleanCap AG Reagent): 11-30

Kozak sequence (including start codon): 79-87

Human IL-1Ra isoform II: 88-624

2xGSSG linker: 625-648

HiBiT: 649-681

Stop codon: 682-684

```
[CAGTGAATTGTAATACGACTCACTATAAGGCGAATTAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGA
CACCGGTCgccaccATGGCTCTGGCCGACCTGTATGAAGAAGGTGGCGGCGGAGGCGGAGAGGGCGAAGAT
AATGCCGACAGCAAAGAGACAATCTGCAGACCCAGCGGCCGGAAGTCCTCTAAGATGCAGGCCTTCAGA
ATCTGGGACGTGAACCAGAAAACCTTCTACCTGCGGAACAATCAGCTGGTGGCCGGCTATCTGCAGGGCC
CCAATGTGAACCTGGAAGAGAAGATCGACGTGGTGCCATCGAGCCCCACGCTCTGTTTCTTGGAATCCA
CGGCGGCAAGATGTGCCTGAGCTGTGTGAAGTCTGGCGACGAGACACGGCTGCAGCTGGAAGCCGTGAA
CATCACCGACCTGAGCGAGAACCGGAAGCAGGACAAGAGATTGCCTTCATCAGAAGCGACAGCGGCC
CACCACAAGCTTTGAGTCTGCTGCTTGCCCTGGCTGGTTCTGTGTACAGCCATGGAAGCCGACCAGCCT
GTGTCTCTGACCAACATGCCTGACGAGGGCGTGATGGTCACCAAGTTCTACTTCCAAGAGGACGAGGGCA
GCAGCGGCGGCAGCAGCGGCGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCTGAATCTAGACCTTCTG
CGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCT
GAGTAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA]
```