**Supplementary Tables**

**Senecavirus A induces mitophagy to promote self-replication through direct interaction of 2C protein with K27-linked ubiquitinated TUFM catalyzed by RNF185**

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**Table S1.** Primers used for construction of recombinant plasmids expressing HA-tagged gene fragments of SVA

|  |  |  |  |
| --- | --- | --- | --- |
| **Plasmid names** | **Primers** | **Primer sequences (5’-3’)** | **Restriction enzymes** |
| pCAGGS-HA-L | L-F | CCGGAATTCATGCAGAACTCTCATTTTTCTTTCGATACAGC | EcoR I |
| L-R | CCGCTCGAGTTACTGTAGTTCGTATACGATGTCCAGTCC | Xho I |
| pCAGGS-HA-VP4 | VP4-F | CGGGGTACCGGTAATGTTCAGACAACCTCAAAGAATG | Kpn I |
| VP4-R | CCGCTCGAGTTATTTGAGGTAGCCAAGAGGGTTCAAG | Xho I |
| pCAGGS-HA-VP2 | VP2-F | CCGGAATTCGATCACAATACCGAAGAAATGGAAAACTC | EcoR I |
| VP2-R | CCGCTCGAGTTACTGTTCCTCGTCCGTCCCG | Xho I |
| pCAGGS-HA-VP3 | VP3-F | CCGGAATTCGGGCCCATTCCCACAGCAC | EcoR I |
| VP3-R | CCGCTCGAGTTAGTGGAACACGTAGGAAGGATTACAATC | Xho I |
| pCAGGS-HA-VP1 | VP1-F | CGGGGTACCTCCACCGACAACGCCGAGAC | Kpn I |
| VP1-R | CCGCTCGAGTTATTGCATCAGCATCTTCTGCTTGTAGCTG | Xho I |
| pCAGGS-HA-2AB | 2AB-F | CCGGAATTCTCAGGCGACATCGAGACCAACC | EcoR I |
| 2AB-R | CCGGCTAGCTTATTGCATCTTGAACAGCTTTCGG | Nhe I |
| pCAGGS-HA-2B | 2B-F | CCGGAATTCCCTGCTTCTGACAACCCAATCTTG | EcoR I |
| 2B-R | CGGGGTACCTTATTGCATCTTGAACAGCTTTCGGAATTTCG | Kpn I |
| pCAGGS-HA-2C | 2C-F | CCGGAATTCGGACCCATGGACACAGTCAAAG | EcoR I |
| 2C-R | CCGCTCGAGTTACTGTAGAACCAGAGTCTGCATATTTCG | Xho I |
| pCAGGS-HA-3A | 3A-F | CCGGAATTCAGCCCTAACGAGAACGACGAC | EcoR I |
| 3A-R | CCGCTCGAGTTACTCGCTCCTAGGCGCTTTAGC | Xho I |
| pCAGGS-HA-3AB | 3AB-F | CCGGAATTCAGCCCTAACGAGAACGACGAC | EcoR I |
| 3AB-R | CCGCTCGAGTTATTGCATTTCCATAAGAGAGAGCGCTC | Xho I |
| pCAGGS-HA-3C | 3C-F | CCGGAATTCCAGCCCAACGTGGACATGG | EcoR I |
| 3C-R | CCGCTCGAGTTATTGCATTGTAGCCAGAGGCTCAC | Xho I |
| pCAGGS-HA-3D | 3D-F | CCGGAATTCGGACTGATGACTGAGCTAGAGCC | EcoR I |
| 3D-R | CGGGGTACCTTATCAGTCGAACAAGGCCCTCCATC | Kpn I |

F: forward primer; R: reverse primer. Restriction sites are underlined.

**Table S2.** Primers used for construction of recombinant plasmids expressing TUFM or ubiquitination-related proteins (SMURF1, RPS27A, UBC, UBB, MARCHF5 and RNF185)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Plasmid names** | **Primers** | **Primer sequences (5'-3')** | **Restriction enzymes** | **GenBank No.** |
| p3×Flag-CMV-TUFM | TUFM(Flag)-F | CCCAAGCTTATGGCGGCCGCTACGTTATTG | Hind Ⅲ | XM\_005064429.4 |
| TUFM(Flag)-R | CGCGGATCCTCAGCTCCACTTGATGTTCTTG | BamH Ⅰ |
| pEGFP-N2-TUFM | TUFM(GFP)-F | CCGCTCGAGCTATGGCGGCCGCTACGTTATTG | Xho Ⅰ |
| TUFM(GFP)-R | CGCGGATCCTCAGCTCCACTTGATGTTCTTGTCCTC | BamH Ⅰ |
| pET-28a-Strep Ⅱ-TUFM | Strep Ⅱ-TUFM-F | CCGGGATCCTGGAGCCACCCGCAGTTCGAAAAAGCGGCCGCTACGTTATTGC | BamH Ⅰ |
| Strep Ⅱ-TUFM-R | CCGCTCGAGGCTCCACTTGATGTTCTTGTCC | Xho Ⅰ |
| pCMV-MYC-SMURF1 | SMURF1-F | CCGGAATTCGAATGAATCACCAGTGCCAACTCAAGGAG | EcoR Ⅰ | XM\_040751332.1 |
| SMURF1-R | CGGGGTACCTTCACTCCACAGCAAAGCCACAGG | Kpn Ⅰ |
| pCMV-MYC-RPS27A | RPS27A-F | CCGGAATTCGAATGCAGATTTTCGTGAAGACCCTCACGG | EcoR Ⅰ | XM\_005070347.4 |
| RPS27A-R | CGGGGTACCTCTACTTGTCCTCTGGTTTGTTGAAGCAGTAAGTC | Kpn Ⅰ |
| pCMV-MYC-UBC | UBC-F | CCGGAATTCGAATGCAGATCTTCGTGAAAACCTTAACTGG | EcoR Ⅰ | XM\_021233330.2 |
| UBC-R | CGGGGTACCTTCAAGCCCAAGAACATGCACAAGGC | Kpn Ⅰ |
| pCMV-MYC-UBB | UBB-F | CCGGAATTCGAATGCAGATCTTCGTGAAGACCCTGACC | EcoR Ⅰ | XM\_040748839.1 |
| UBB-R | CGGGGTACCTTTAATAGCCACCCCTGAGGCGGAG | Kpn Ⅰ |
| pCMV-MYC-MARCHF5 | MARCHF5-F | CCGGAATTCGAATGCCGGACCAAGCCCTTC | EcoR Ⅰ | XM\_005063651.4 |
| MARCHF5-R | CGGGGTACCTTTATGCTTCTTCTTGCTCTGGATAATTTAGG | Kpn Ⅰ |
| pCMV-MYC-RNF185 | RNF185(MYC)-F | CCGGAATTCGAATGGCAAGTAAGGGGCCCTCGACCTC | EcoR Ⅰ | XM\_040746844.1 |
| RNF185(MYC)-R | CGGGGTACCTTTAGGCGATCAGGAGCCAGAAC | Kpn Ⅰ |
| pCAGGS-HA-RNF185 | RNF185(HA)-F | CCGGAATTCATGGCAAGTAAGGGGCCCTC | EcoR Ⅰ |
| RNF185(HA)-R | CGGGGTACCATTAGGCGATCAGGAGCCAGAAC | Kpn Ⅰ |

F: forward primer; R: reverse primer. Restriction sites are underlined.

**Table S3**. Primers used for construction of recombinant plasmids expressing each of the truncated TUFM, 2C and RNF185 proteins

|  |  |  |
| --- | --- | --- |
| **Plasmid names** | **Primers** | **Primer sequences (5'-3')** |
| p3×Flag-CMV-TUFM ∆Ⅰ | TUFM 252-F | gattacaaggatgacgatgacAAGCTTATGGACCTGGAGAAGCCCTTTCTGC |
| TUFM 452-R | gtcacagggatgccacccgGGATCCTCAGCTCCACTTGATGTTCTTGTCCTCCTC |
| p3×Flag-CMV-TUFM ∆Ⅱ | TUFM 1-F | gattacaaggatgacgatgacAAGCTTATGGCGGCCGCTACGTTATTGCG |
| TUFM 275-R | ctggtggggctggatggaCACCACTGTGCCCCGGCC |
| TUFM 344-F | TCCATCCAGCCCCACCAGAAGGTG |
| TUFM 452-R | gtcacagggatgccacccgGGATCCTCAGCTCCACTTGATGTTCTTGTCCTCCTC |
| p3×Flag-CMV-TUFM ∆Ⅲ | TUFM 1-F | gattacaaggatgacgatgacAAGCTTATGGCGGCCGCTACGTTATTGCG |
| TUFM 349-R | gatgttcttgtcctcctcGTGGGGCTGGATGGAGCCTGGC |
| TUFM 443-F | gaggaggacaagaacatcAAGTGGAGCTAAGGATCCCGGGTGGCATCCCTGTGAC |
| TUFM 452-R | gtcacagggatgccacccgGGATCCTTAGCTCCACTTGATGTTCTTGTCCTCCTC |
| p3×Flag-CMV-TUFM aa1–252 | TUFM 1-F | gattacaaggatgacgatgacAAGCTTATGGCGGCCGCTACGTTATTGCG |
| TUFM 252-R | cagggatgccacccgGGATCCTTACCGGGTGGGGACCGGTATG |
| p3×Flag-CMV-TUFM aa1-55 | TUFM 1-F | gattacaaggatgacgatgacAAGCTTATGGCGGCCGCTACGTTATTGCG |
| TUFM 55-R | cagggatgccacccgGGATCCTTATTACTTGTCGCGCACGTAGGTC |
| p3×Flag-CMV-TUFM aa56–252 | TUFM 56-F | gattacaaggatgacgatgacAAGCTTATGCCCCATGTGAATGTGGGTAC |
| TUFM 252-R | cagggatgccacccgGGATCCTTACCGGGTGGGGACCGGTATG |
| p3×Flag-CMV-TUFM aa275–452 | TUFM 275-F | gattacaaggatgacgatgacAAGCTTGTGACCGGTACACTAGAGCG |
| TUFM 452-R | gtcacagggatgccacccgGGATCCTCAGCTCCACTTGATGTTCTTGTCCTCCTC |
| p3×Flag-CMV-TUFM aa275–344 | TUFM 275-F | gattacaaggatgacgatgacAAGCTTGTGACCGGTACACTAGAGCG |
| TUFM 344-R | cagggatgccacccgGGATCCTTATCAGCCTGGCTTGACCATGACC |
| p3×Flag-CMV-TUFM aa345–452 | TUFM 345-F | gattacaaggatgacgatgacaAGCTTATGTCCATCCAGCCCCACCAG |
| TUFM 452-R | gtcacagggatgccacccgGGATCCTCAGCTCCACTTGATGTTCTTGTCCTCCTC |
| pCAGGS-HA-2C  aa1–175 | 2C 1-F | gatgttccagattacgctGAATTCGGACCCATGGACACAGTCAAAGAC |
| 2C 175-R | ctagctcgagcatgcccgGGTACCATTATCCCAGATCGTCCATCAAGGTTAC |
| pCAGGS-HA-2C  aa1–69 | 2C 1-F | gatgttccagattacgctGAATTCGGACCCATGGACACAGTCAAAGAC |
| 2C 69-R | ctagctcgagcatgcccgGGTACCATTAAGAACCGGCCATGAAGGCATC |
| pCAGGS-HA-2C aa70–175 | 2C 70-F | gatgttccagattacgctGAATTCGGGCCCCCTCTTGGTGAC |
| 2C 175-R | ctagctcgagcatgcccgGGTACCATTATCCCAGATCGTCCATCAAGGTTAC |
| pCAGGS-HA-2C aa176–321 | 2C 176-F | gatgttccagattacgctGAATTCCAAAACCCGGATGGGCAAGATTTC |
| 2C 321-R | ctagctcgagcatgcccgGGTACCATTACTGTAGAACCAGAGTCTGCATATTTCGG |
| pCAGGS-HA-2C aa212–321 | 2C 212-F | gatgttccagattacgctGAATTCAATCTTATCATTGCAACTACAAACCTCCCTC |
| 2C 321-R | ctagctcgagcatgcccgGGTACCATTACTGTAGAACCAGAGTCTGCATATTTCGG |
| pCAGGS-HA-2C aa231–321 | 2C 231-F | gatgttccagattacgctGAATTCGATCCTTCTGCAGTCTCTCGGC |
| 2C 321-R | ctagctcgagcatgcccgGGTACCATTACTGTAGAACCAGAGTCTGCATATTTCGG |
| pCAGGS-HA-2C aa256–321 | 2C 256-F | gatgttccagattacgctCTGAATTTTGACCTGGCTTTCAGACGC |
| 2C 321-R | ctagctcgagcatgcccgGGTACCATTACTGTAGAACCAGAGTCTGCATATTTCGG |
| pCAGGS-HA-2C aa285–321 | 2C 285-F | gatgttccagattacgctCGCTTCAAAAATGGTCATCAAAGCTTC |
| 2C 321-R | ctagctcgagcatgcccgGGTACCATTACTGTAGAACCAGAGTCTGCATATTTCGG |
| pCAGGS-HA-RNF185 ∆2–18 | RNF185 18-F | cgatgttccagattacgctGAATTCATGGGCCCTAGTGGCAGCAGCAATG |
| RNF185 192-R | GCTagctcgagcatgcccgGGTACCATTAGGCGATCAGGAGCC |
| pCAGGS-HA-RNF185 ∆19–38 | RNF185 1-F | cgatgttccagattacgctGAATTCATGGCAAGTAAGGGGC |
| RNF185 18-R | atatattgcaccctgcacttGAATTCTCAGTGGATG |
| RNF185 39-F | aagtgcagggtgcaatatatGCCTGGACACAGCC |
| RNF185 192-R | gctagctcgagcatgcccgGGTACCATTAGGCGATCAGGAGCC |
| pCAGGS-HA-RNF185 ∆39–80 | RNF185 1-F | cgatgttccagattacgctGAATTCATGGCAAGTAAGGGGC |
| RNF185 38-R | attccagcctcgaaggtaCTGTCCTGC |
| RNF185 81-F | taccttcgaggctggaatCAGCCGGGAC |
| RNF185 192-R | gctagctcgagcatgcccgGGTACCATTAGGCGATCAGGAGCC |
| pCAGGS-HA-RNF185 ∆81–132 | RNF185 1-F | cgatgttccagattacgctGAATTCATGGCAAGTAAGGGGC |
| RNF185 80-R | catctgaaatttgcaaacTGGACACACTTGTCTG |
| RNF185 133-F | gtttgcaaatttcagatgTCTTTTGGAATTGGAGC |
| RNF185 192-R | gctagctcgagcatgcccgGGTACCATTAGGCGATCAGGAGCC |
| pCAGGS-HA-RNF185  ∆133–191 | RNF185 1-F | cgatgttccagattacgctGAATTCATGGCAAGTAAGGGGC |
| RNF185 132-R | ctagctcgagcatgcccgGGTACCATTAGCCACCATCTCCAAATCC |
| pCAGGS-HA-RNF185  ∆155–191 | RNF185 1-F | cgatgttccagattacgctGAATTCATGGCAAGTAAGGGGC |
| RNF185 154-R | gctagctcgagcatgcccgGGTACCAATTTATGTTAAATGCTGTGGCAAATAT |
| pCAGGS-HA-RNF185  ∆172–191 | RNF185 1-F | cgatgttccagattacgctGAATTCATGGCAAGTAAGGGGC |
| RNF185 171-R | gctagctcgagcatgcccgGGTACCATTACTCATCCACGTACTGGGGTG |

F: forward primer; R: reverse primer. Homologous arm sequences are shown in lowercase font.

**Table S4**. Primers used for construction of recombinant plasmids expressing TUFM, 2C and Ub containing a single-site mutation

|  |  |  |
| --- | --- | --- |
| **Plasmid names** | **Primers** | **Primer sequences (5'-3')** |
| p3×Flag-CMV-TUFM E193A | TUFM E193A-F | GGACTCAGAGATGGTGG**CA**CTGGTGGAGCTGGAGATC |
| TUFM E193A-R | GATCTCCAGCTCCACCAG**TG**CCACCATCTCTGAGTCC |
| p3×Flag-CMV-TUFM E196A | TUFM E196A-F | GTGGAGCTGGTGG**CA**CTGGAGATCCGG |
| TUFM E196A-R | CCGGATCTCCAG**TG**CCACCAGCTCCAC |
| p3×Flag-CMV-TUFM E211A | TUFM E211A-F | GGATATAAAGGAG**C**AGAGGCCCCAGTC |
| TUFM E211A-R | GACTGGGGCCTCT**G**CTCCTTTATATCC |
| p3×Flag-CMV-TUFM E212A | TUFM E212A-F | GGATATAAAGGAGAAG**CC**GCCCCAGTCATCATAG |
| TUFM E212A-R | CTATGATGACTGGGGC**GG**CTTCTCCTTTATATCC |
| p3×Flag-CMV-TUFM R200A | TUFM R200A-F | CTGGTGGAGCTGGAGATC**GCA**GAGCTGCTCACCGAGTTTG |
| TUFM R200A-R | CAAACTCGGTGAGCAGCTC**TGC**GATCTCCAGCTCCACCAG |
| pCAGGS-HA-2C N240A | 2C N240A-F | GCAGTCTCTCGGCGTATC**GC**CTACGACCTGACTCTAG |
| 2C N240A-R | CTAGAGTCAGGTCGTAG**GC**GATACGCCGAGAGACTGC |
| pCAGGS-HA-2C R286A | 2C R286A-F | GTGGACGTGGCTGTG**GC**CTTCAAAAATGGTC |
| 2C R286A-R | GACCATTTTTGAAG**GC**CACAGCCACGTCCAC |
| pCAGGS-HA-2C S293A | 2C S293A-F | CTTCAAAAATGGTCATCAA**GC**CTTCAATCTCCTAGAGTTG |
| 2C S293A-R | CAACTCTAGGAGATTGAAG**GC**TTGATGACCATTTTTGAAG |
| pCAGGS-HA-2C R308A | 2C R308A-F | CTATTTGTGCAGACATT**GC**GGCCAAGCAACAAGGTG |
| 2C R308A-R | CACCTTGTTGCTTGGCC**GC**AATGTCTGCACAAATAG |
| pCAGGS-HA-2C D242A | 2C D242A-F | GCGTATCAACTACG**C**CCTGACTCTAGAAG |
| 2C D242A-R | CTTCTAGAGTCAGG**G**CGTAGTTGATACGC |
| pCAGGS-HA-2C K252A | 2C K252A-F | GTATCTGAGGCCTAC**GCA**AAGCACACACGGCTG |
| 2C K252A-R | CAGCCGTGTGTGCTT**TGC**GTAGGCCTCAGATAC |
| pCAGGS-HA-2C H277A | 2C H277A-F | CATTTATCCTTTTGCTGCC**GCA**GTGCCCTTCGTGGACGTG |
| 2C H277A-R | CACGTCCACGAAGGGCAC**TGC**GGCAGCAAAAGGATAAATG |
| pCAGGS-HA-2C D282A | 2C D282A-F | CATGTGCCCTTCGTGG**C**CGTGGCTGTGC |
| 2C D282A-R | GCACAGCCACG**G**CCACGAAGGGCACATG |
| pCAGGS-HA-2C K288A | 2C K288A-F | GTGGCTGTGCGCTTC**GC**AAATGGTCATCAAAG |
| 2C K288A-R | CTTTGATGACCATTT**GC**GAAGCGCACAGCCAC |
| pCAGGS-HA-2C H291A | 2C H291A-F | GCGCTTCAAAAATGGT**GC**TCAAAGCTTCAATCTCC |
| 2C H291A-R | GGAGATTGAAGCTTTGA**GC**ACCATTTTTGAAGCGC |
| pCAGGS-HA-2C E298A | 2C E298A-F | CAAAGCTTCAATCTCCTAG**C**GTTGGTCGACTCTATTTG |
| 2C E298A-R | CAAATAGAGTCGACCAAC**G**CTAGGAGATTGAAGCTTTG |
| pCAGGS-HA-2C D301A | 2C D301A-F | CCTAGAGTTGGTCG**C**CTCTATTTGTGCAG |
| 2C D301A-R | CTGCACAAATAGAG**G**CGACCAACTCTAGG |
| pCAGGS-HA-2C D306A | 2C D306A-F | CTCTATTTGTGCAG**C**CATTCGGGCCAAG |
| 2C D306A-R | CTTGGCCCGAATG**G**CTGCACAAATAGAG |
| pCMV-MYC-Ub K6R | Ub K6R-F | GCAGATCTTCGTGA**GA**ACCCTGACCGGCAAG |
| Ub K6R-R | CTTGCCGGTCAGGGT**TC**TCACGAAGATCTGC |
| pCMV-MYC-Ub K11R | Ub K11R-F | GAAGACCCTGACCGGCA**GA**ACCATCACCCTGGAGG |
| Ub K11R-R | CCTCCAGGGTGATGGT**TC**TGCCGGTCAGGGTCTTC |
| pCMV-MYC-Ub K27R | Ub K27R-F | CATCGAAAACGTCA**G**GGCCAAGATCCAGG |
| Ub K27R-R | CCTGGATCTTGGCC**C**TGACGTTTTCGATG |
| pCMV-MYC-Ub K29R | Ub K29R-F | GAAAACGTCAAGGCCA**G**GATCCAGGATAAGGAG |
| Ub K29R-R | CTCCTTATCCTGGATC**C**TGGCCTTGACGTTTTC |
| pCMV-MYC-Ub K33R | Ub K33R-F | CAAGATCCAGGATA**GA**GAGGGCATCCCCCC |
| Ub K33R-R | GGGGGGATGCCCTC**TC**TATCCTGGATCTTG |
| pCMV-MYC-Ub K48R | Ub K48R-F | CATCTTTGCAGGCA**G**GCAGCTGGAAGATG |
| Ub K48R-R | CATCTTCCAGCTGC**C**TGCCTGCAAAGATG |
| pCMV-MYC-Ub K63R | Ub K63R-F | GATTACAACATCCAGA**GG**GAGTCTACCCTGCACC |
| Ub K63R-R | GGTGCAGGGTAGACTC**CC**TCTGGATGTTGTAATC |

F: forward primer; R: reverse primer. The nucleotides in the primer sequences used for introducing a single-site mutation are marked in bold.