**Supplementary Materials**

**AIMP2 restricts EV71 replication by recruiting SMURF2 to promote the degradation of 3D polymerase**

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**Table S1.** All PCR and qPCR Primers, siRNAs used in this study.

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| **Primer name** | **Sequence of oligonucleotide (5’-3’)** |
| PCDH-CMV-SF-AIMP2-F | GATCTCGAGCTCAAGCTTCGAATTCATGCCGATGTACCAGGTAAAGC |
| PCDH-CMV-SF-AIMP2-R | CTGAGGGTGGCTCCAGGATCCCTTAAGGAGCTTGAGGGCCG |
| PCMV-HA-AIMP2-F | TTATGGCCATGGAGGCCCGAATTCGGATGCCGATGTACCAGGTAAAGC |
| PCMV-HA-AIMP2-R | TCCCCGCGGCCGCGGTACCTCGAGATCACTTAAGGAGCTTGAGGGC |
| PCMV-Myc-SMURF2-F | GCTCTAGCCCGGGCGGATCCATGTCTAACCCCGGAGGC |
| PCMV-Myc-SMURF2-R | GTCGACGGTATCGATAAGCTTTTCCACAGCAAATCCACATG |
| PCMV-Myc-CBL-F | GCTCTAGCCCGGGCGGATCC ATGGCCGGCAACGTGAAG |
| PCMV-Myc-CBL -R | GTCGACGGTATCGATAAGCTT GGTAGCTACATGGGCAGGAGAAG |
| PCMV-Myc-ITCH-F | GCTCTAGCCCGGGCGGATCCATGTCTGACAGTGGATCACAAC |
| PCMV-Myc-ITCH-R | GTCGACGGTATCGATAAGCTTCTCTTGTCCAAATCCTTCTGT |
| PCMV-Myc-TRAF2-F | GCTCTAGCCCGGGCGGATCCATGGCTGCAGCTAGCGTGAC |
| PCMV-Myc-TRAF2-R | GTCGACGGTATCGATAAGCTTGAGCCCTGTCAGGTCCACA |
| PCMV-Myc-TRAF3-F | GCTCTAGCCCGGGCGGATCCATGGAGTCGAGTAAAAAGATGG |
| PCMV-Myc-TRAF3-R | GTCGACGGTATCGATAAGCTTGGGATCGGGCAGATCCGA |
| PCMV-Myc-TRAF6-F | GCTCTAGCCCGGGCGGATCCATGAGTCTGCTAAACTGTGA |
| PCMV-Myc-TRAF6-R | GTCGACGGTATCGATAAGCTTTACCCCTGCATCAGTACTTCG |
| PCDH-CMV-SF-SMURF2-F | ATCTCGAGCTCAAGCTTCGAATTCATGTCTAACCCCGGAGGCC |
| PCDH-CMV-SF-SMURF2-R | CTGAGGGTGGCTCCAGGATCCTCATTCCACAGCAAATCCACATG |
| p3×Flag-CMV-3C-F | ATTAAGCTTGCGGCCGCGAATTCCGGAGGATGGGCCCGAGCCTTGACTT |
| p3×Flag-CMV-3C-R | GGTCTTTGTAGTCAGCCCGGGATCCTTGTTCACTAGCAAAGTAACTCC |
| qPCR-human AIMP2-F | AGGTAAAGCCCTATCACGG |
| qPCR-human AIMP2-F | ACAGGTTAGACTCTTCCTGCAC |
| qPCR-EV71-F | TGAATGCGGCTAATCCCAACT |
| qPCR-EV71-R | AAGAAACACGGACACCCAAAG |
| qPCR-GAPDH-F | CCCACTCCTCCACCTTTGACG |
| qPCR-GAPDH-R | CACCACCCTGTTGCTGTAGCCA |
| qPCR-EV713C-F | CATCAGGCAAGTCCAAAC |
| qPCR-EV713C-R | GTGTCAAGAGTAATGAGGGT |
| qPCR-EV713D-F | GGCATTGATTTGGATGAACT |
| qPCR-EV713D-R | CATTGGCATAGTAGGGTGAA |
| Negative control siRNA-F | UUCUCCGAACGUGUCACGU |
| Negative control siRNA-R | ACGUGACAGGUUCGGAGAA |
| siAIMP2#1-F | UGGACUUGAAUUCAGUGCUUGGGAA |
| siAIMP2#1-R | UUCCCAAGCACUGAAUUCAAGUCCA |
| siAIMP2#2-F | GGAUGAGCCCACGACUUUA |
| siAIMP2#2-R | UAAAGUCGUGGGCUCAUCC |
| siSMURF2-F | GAUCUCUGGAAGCGAUUAA |
| siSMURF2-R | UUAAUCGCUUCCAGAGAUC |

**Supplementary Figures**

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**Figure. S1** AIMP2 inhibits the infection of enterovirus serotypes CVA16 and CVB1. **A, B** RD cells were transfected with the AIMP2-Flag plasmid for 24 h and were then infected with CVA16 (**A**) and CVB1 (**B**) (MOI =1) for 12 h. Virus in cell supernatants was evaluated by a plaque formation assay. The results represent the means ± SD (error bars) from three independent experiments. Statistical significance was analyzed using Student's t-test (\*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001).

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**Figure. S2** The EV71 3C protease downregulates AIMP2. **A, B** HEK293T cells were transfected with increasing amounts of the 3C-Flag plasmid and HA-AIMP2 plasmid for 36 h. The protein expression of 3C protease and AIMP2 was measured by immunoblotting with the indicated antibodies (**A**). The RNA levels were determined by RT‒qPCR (**B**).



**Figure. S3** AIMP2 does not interact with EV71 helicase 2C.HEK293T cells were transfected with the HA-AIMP2 plasmid alone, the 2C-Flag plasmid alone or SMURF2-Myc in combination with AIMP2-Flag plasmid. After 36 h, the cell lysates were immunoprecipitated with M2 beads and subjected to western blot analysis with the indicated antibodies.