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Supplementary Data

C1QTNF5 is a novel attachment factor that facilitates the entry of influenza A virus

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

Gene	Purpose	Sequence (5'-3')
pCMV-HA-C1QTNF5	Cloning	F-CCGGAATTCATATGAGGCCACTCCTCGTCCTG
		R-CGGGGTACCCTAAGCAAAGACTGGGGAGCTGTG
pCMV-HA-C1QTNF5 (1-103 aa)	Cloning	F-CCGGAATTCATATGAGGCCACTCCTCGTCCTG
		R-CGGGGTACCCTATCGCGGAGGCACCGAG
pCMV-HA-C1QTNF5 (103-243 aa)	Cloning	F-CCGGAATTCATATGTCCGCCTTCAGCGCC
		R-CGGGGTACCCTAAGCAAAGACTGGGGAGCTG
pCDH-Flag-C1QTNF5	Cloning	F-CCGGAATTCATGAGGCCACTCCTCGTCCTG
		R-CGCGGATCCAGCAAAGACTGGGGAGCTGTGC
pCMV-Myc-C1QTNF5	Cloning	F-CGCGGATCCATGAGGCCACTCCTCGTCC
		R-CCCAAGCTTAGCAAAGACTGGGGAGCTGTG
pCMV-Flag-PR8-HA2	Cloning	F-GCGTCGACCATGCTATTTGGAGCCATTGCCG
		R-GGGGTACCTCAGATGCATATTCTGCACTGCAAA
pGEX-4T-C1QTNF5	Cloning	F-CCGGAATTCATGAGGCCACTCCTCGTCC
		R-CGCGTCGACCTAAGCAAAGACTGGGGAGCTG
PET-30A-PR8-HA	Cloning	F-CGCGGATCCATGGACACAATATGTATAGGCTACC
		R-CCGCTCGAGGATGCATATTCTGCACTGCAAAGAT
siNC	Knock down	F-UUCUCCGAACGUGUCACGUTT
		R-ACGUGACACGUUCGGAGAATT
siC1QTNF5	Knock down	F-GCAUCUAUGCCAGCAUCAATT
		R-UUGAUGCUGGCAUAGAUGCTT
sgRNA#1	Knock down	GGACATTACGACGCCGTCAC
sgRNA#2	Knock down	TTGGCGCTGAAGGCGGATCG
Human GAPDH	qPCR	F-AGGTCGGAGTCAACGGATTT
		R-TGACGGTGCCATGGAATTTG
IAV NP-vRNA	RT	GGCCGTCATGGTGGCGAATGAATGGACGGAGAACA AGGATTGC
IAV NP-mRNA	RT	CCAGATCGTTCGAGTCGTTTTTTTTTTTTTTTTCTT TAATTGTC
IAV NP-vRNA	qPCR	F-GGCCGTCATGGTGGCGAAT
		R-CTCAATATGAGTGCAGACCGTGCT
IAV NP-mRNA	qPCR	F-CCAGATCGTTCGAGTCGT
		R-CGATCGTGCCCTCCTTTG
Mouse Actin	qPCR	F-CTAAGGCCAACCGTGAAAAG
		R-ACCAGAGGCATACAGGGACA
EV71	qPCR	F-TGAATGCGGCTAATCCCAACT
		R-AAGAAACACGGACACCCAAAG
Human C1QTNF5	qPCR	F-GTCTACTACTTCGCCGTCCA
		R-GGAGAAGGTGCTGTCTGTCT

Supplementary Figures.

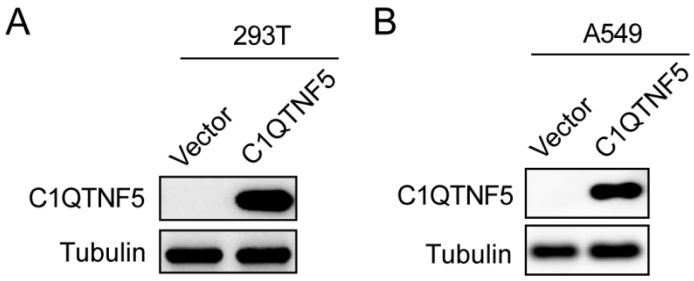

Figure. S1 Construction of C1QTNF5 stable overexpressing cell line. **A** Stable C1QTNF5 overexpressing cell line was constructed with 293T cells and verified by Western blotting. **B** Stable C1QTNF5 overexpressing cell line was constructed with A549 cells and verified by Western blotting.

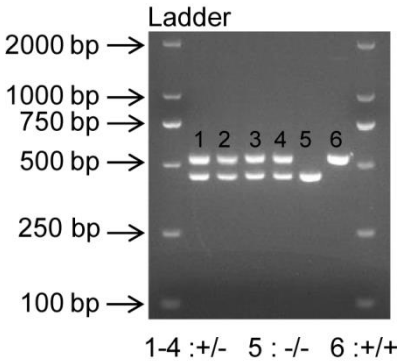

Figure. S2 Identification of mouse genotypes by PCR.

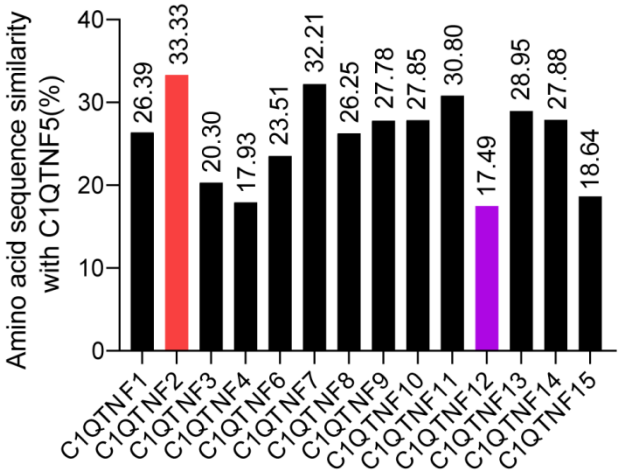

Figure. S3 The amino acid sequence similarity between other members of C1q/TNF family and C1QTNF5. The corresponding sequences of C1QTNF5 and other members of the C1q/TNF family were found in the NCBI database, and the amino acid sequence similarity was analyzed by using DNAMAN software.

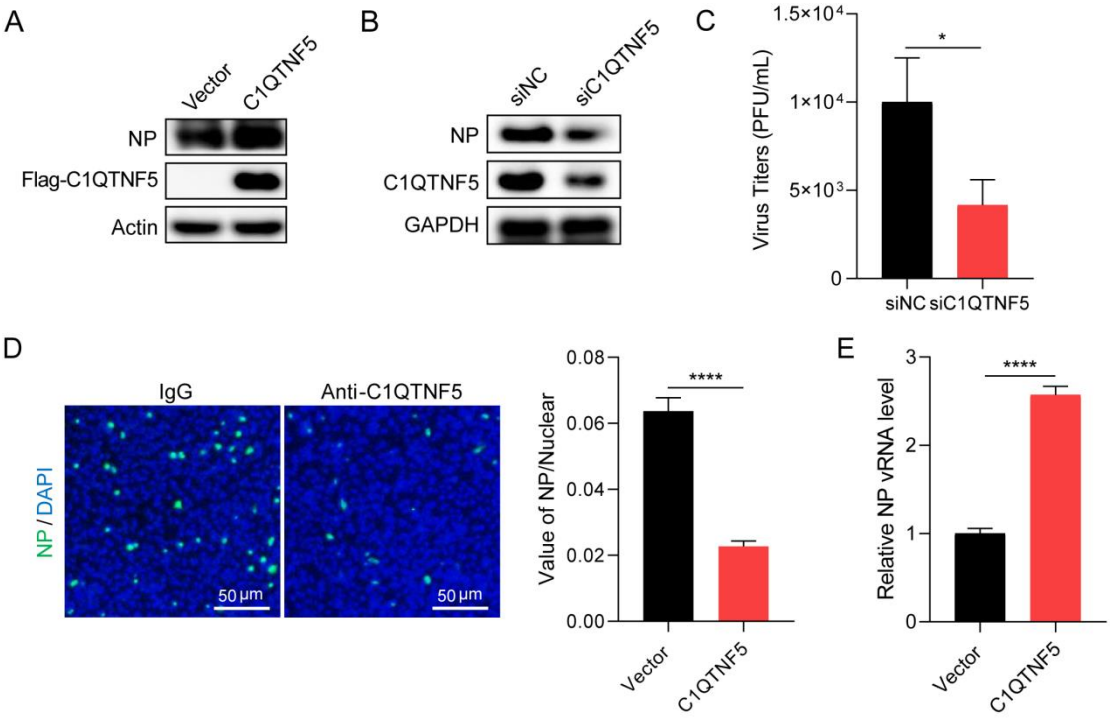

Figure. S4 C1QTNF5 promotes the infection of influenza H3N2 subtype. **A** Overexpression of C1QTNF5 promotes H3N2 infection. C1QTNF5 stable overexpressing A549 cells were infected with H3N2 for 48 h, and cell lysates were detected using the indicated antibodies. **B, C** Knockdown of C1QTNF5 inhibits H3N2 infection. MDCK cells were transfected with siRNAs (siNC or siC1QTNF5) for 48 h and then infected with H3N2 (MOI=0.1) for 12 h. Viral NP protein levels were measured at 12 h by Western blotting (**B**). Viral titer in cell supernatants were determined by plaque assay (**C**). **D** Blocking A549 cells with anti-C1QTNF5 antibody inhibits H3N2 subtype entry. A549 cells were incubated with anti-C1QTNF5 antibody (10 μg/mL) or control IgG at 4 ℃ for 2 h, and then were incubated with H3N2 (MOI=5) for 1 h at 4 ℃. Immediately cells were washed three times with PBS and were cultured at 37 ℃ for another 12 h, a immunofluorescence assay was performed, and then visualized by fluorescence microscopy (NP:green; Nuclear: blue), scale bar represents 50 μm, the fluorescence area was analyzed using image J software to perform statistical analysis. **E** C1QTNF5 promotes the attachment of H3N2. C1QTNF5 stable overexpressing A549 cells were infected with H3N2 (MOI=2) for 1 h at 4 ℃. Immediately washing three times with PBS, the cells were lysed and the attachment difference of H3N2 was assessed by detecting viral NP vRNA levels. The

data were shown as mean ± SD from three independent experiments. *, P < 0.05; ****, P < 0.0001.

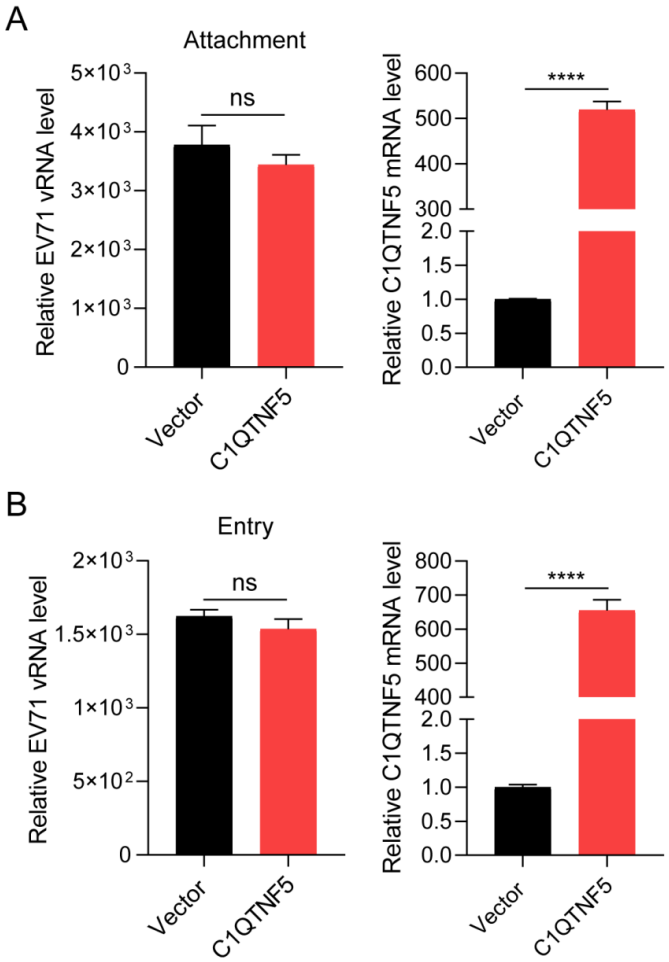

Figure. S5 C1QTNF5 does not affect the attachment and entry of EV71. **A** Attachment assay, RD cells were transfected with 1.5 μg Flag-C1QTNF5 expression plasmid for 24 h and incubated with EV71 virus (MOI=3) for 1 h at 4 ℃. PBS washed the cells three times to remove the unattached virions. EV71 RNA levels were detected by qPCR in cell lysates. **B** Entry assay, RD cells were transfected with 1.5 μg Flag-C1QTNF5 expression plasmid for 24 h and incubated with EV71 virus (MOI=3) for 1 h at 4 ℃. PBS washed the cells three times to remove the unattached virions, and the infected cells were cultured at 37 ℃ for another 1 h. Then, PBS washed the cells three times again to remove the attached but not internalized virions. The cells were lysed and the entry of EV71 was assessed by detecting viral RNA levels.