Supplementary information

The deubiquitinase OTUD1 influences HIV-1 release by regulating the host restriction factor BST-2

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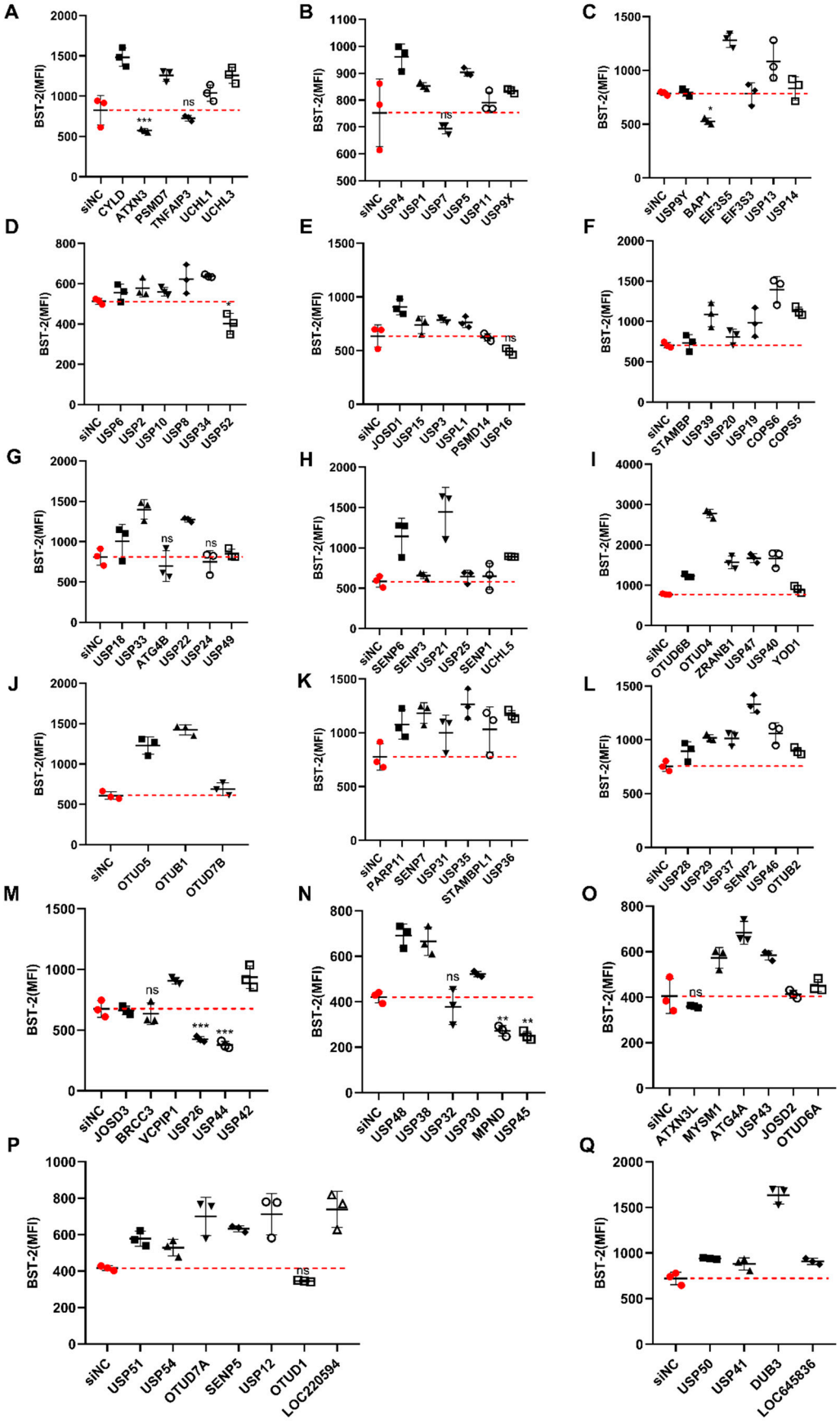


Figure S1. Screening of DUB-siRNA libraries that regulate BST-2 expression levels on membrane surfaces (The first round). HeLa cells were transfected with siNC and 98 DUB-siRNA for 48 h, then the surface BST-2 expression level was detected by flow cytometry. Data are presented as mean ± standard deviation (SD). (two-tailed paired t-test). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

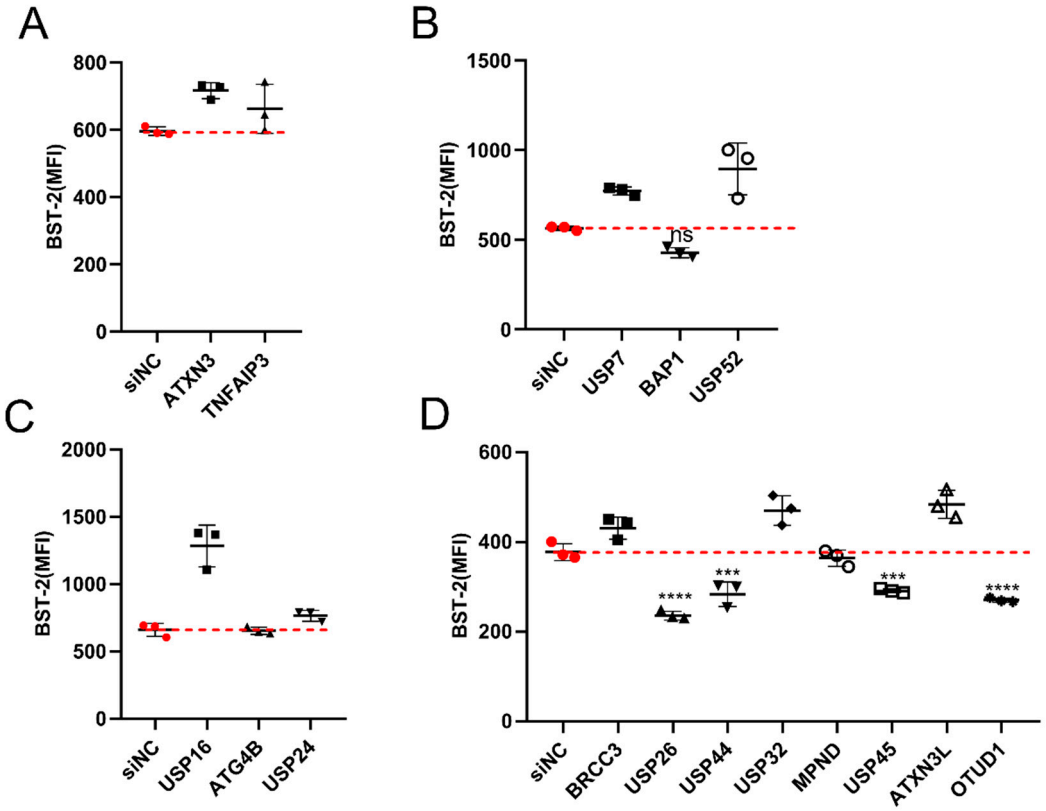


Figure S2. Screening of DUB-siRNA libraries that regulate BST-2 expression levels on membrane surfaces (The second round). HeLa cells were transfected with siNC and 16 DUB-siRNA for 48 h, then the surface BST-2 expression level was detected by flow cytometry. Data are presented as mean ± standard deviation (SD). (two-tailed paired t-test). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

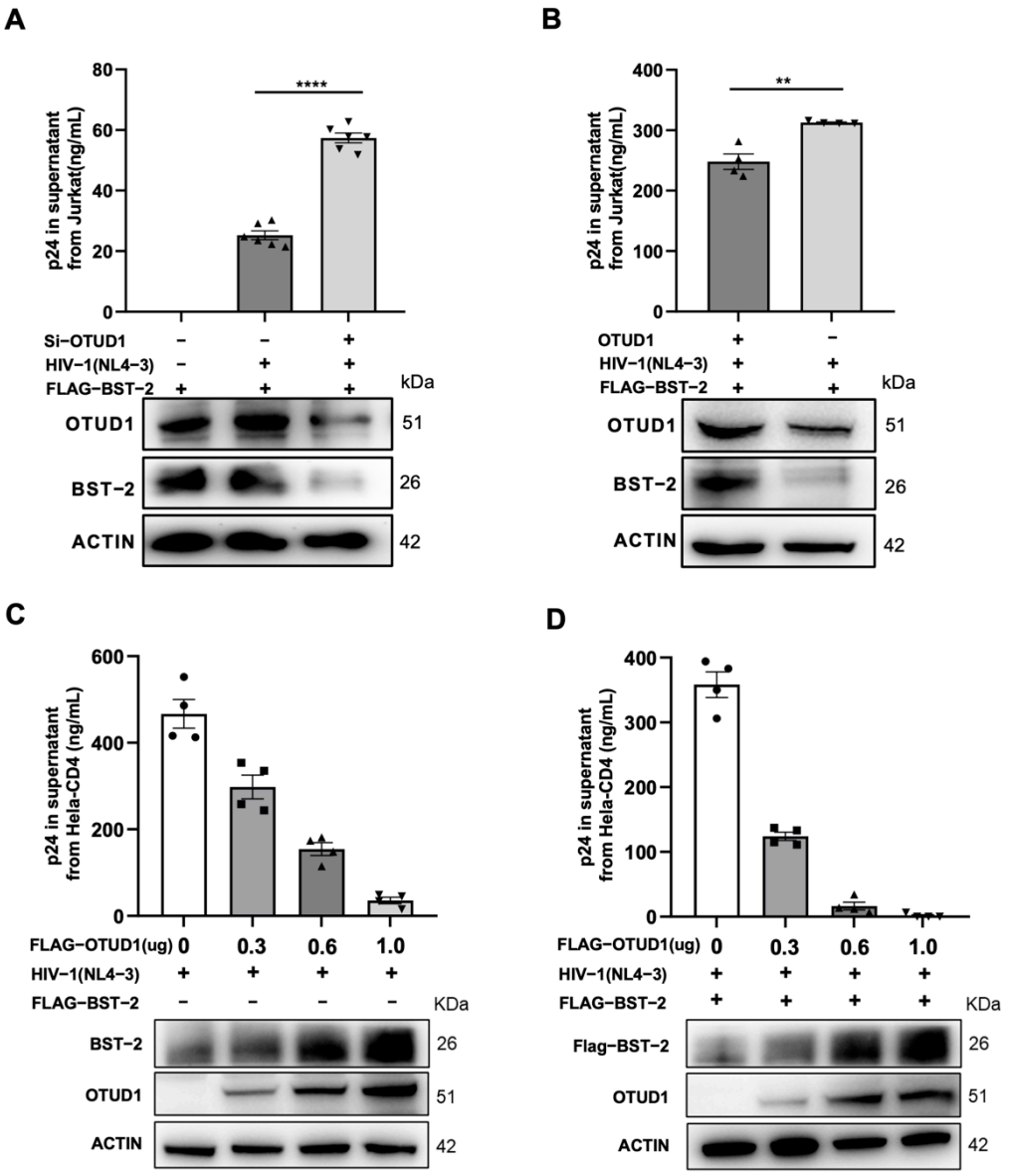


Figure S3. OTUD1 inhibits HIV-1 release by influencing BST-2 expression in Jurkat and Hela-CD4 cells. (A) Jurkat cells were transfected with OTUD1-specific siRNA, FLAG-BST-2 and pro-HIV-1 for 48 h, then the supernatant protein p24 was detected by ELISA, and the expression level of BST-2 in the cells was detected by western blotting. (B) Jurkat cells were transfected with OTUD1, FLAG-BST-2 and pro-HIV-1 for 48 h, then the supernatant protein p24 was detected by ELISA, and the expression level of BST-2 in the cells was detected by western blotting. (C-D) Hela-CD4 cells were transfected with different doses of OTUD1, with or without FLAG-

BST-2 for 24 h, then infected with HIV-1(NL4-3) for 48h, the supernatant protein p24 was detected by ELISA, and the expression level of BST-2 and OTUD1 in the cells was detected by western blotting. Data are presented as mean ± standard deviation (SD). (two-tailed paired t-test). **$p < 0.01$; ****$p < 0.0001$.

Table S1. Primers related to the gene synthesis

Name	Reference Sequence	Primer Sequence (5′ - 3′)
FLAG-OTUD1-F	NM_001145373.3	AAGCTT ATGCAGCTCTACAGCAGCGTCT
FLAG-OTUD1-R		GAATTCTCAAGAGCATGCATTTTGTTCAATA
FLAG-BST-2-F	NM_004335.4	GAAGATCTATGGCATCTACTTCGTATGACTATT
FLAG-BST-2-R		CGGGATCCTCACTGCAGCAGAGCGCT
OTUD1 C320S mutation-F	NM_001145373.3	CTCGGTAGAGGCTGTTGCCGTCTGGA
OTUD1 C320S mutation-R		TCCAGACGGCAACAGCCTCTACCGAGC
BST-2 K18R mutation-F	NM_004335.4	CTTACAGCGCCTATCCCCGTCTTCCATGGG
BST-2 K18R mutation-R		CCCATGGAAGACGGGGATAGGCGCTGTAAG
BST-2 K21 Rmutation-F		CCCCAGCAGAAGCCTACAGCGCTTATCCCC
BST-2 K21R mutation-R		GGGGATAAGCGCTGTAGGCTTCTGCTGGGG
BST-2 K47R mutation-F		TCGCTGTTGGCCCTGATGGTGAAGATAATCAAGGGC
BST-2 K47R mutation-R		GCCCTTGATTATCTTCACCATCAGGGCCAACAGCGA
BST-2 K79R mutation-F		TGAAAGCCCCTCTGGGCCTCGGTCAGC
BST-2 K79R mutation-R		GCTGACCGAGGCCCAGAGGGGCTTTCA
BST-2 K106R mutation-F		TCCTTGGGCCCTCTCTGCATCCAGGGAAGC
BST-2 K106R mutation-R		GCTTCCCTGGATGCAGAGAGGGCCCAAGGA
BST-2 K111R mutation-F		CAAGCTCCTCCACTTTCCTTTGTCCTTGGGCCTTC
BST-2K111R mutation-R		GAAGGCCCAAGGACAAAGGAAAGTGGAGGAGCTG
BST-2 K112R mutation-F		GCTCCTCCACTCTCTTTTGTCCTTGGGCCTTCT
BST-2 K112R mutation-R		AGAAGGCCCAAGGACAAAAGAGAGTGGAGGAGC
BST-2K126RCmutation-F		GCGTCCTGAAGCCTATGGTTTAATGTAGTGATCTCTC
BST-2 K126R mutation-R		AGAGATCACTACATTAAACCATAGGCTTCAGGACG
BST-2 K151R mutation-F		GCTGGGGTAGTACTTCCTGTCCGCGATTCTCAC
BST-2 K151R mutation-R		GTGAGAATCGCGGACAGGAAGTACTACCCCAGC
BST-2 K152R mutation-F		GAGCTGGGGTAGTACCTCTTGTCCGCGATTC

BST-2 K152R mutation-R		GAATCGCGGACAAGAGGTACTACCCCAGCTC

Table S2. Primers related to RT-qPCR

Name	**Primer Sequence (5′ - 3′)**
OTUD1-Forward primer	TTTATCATCGCTGCTGCCCA
OTUD1-Reverse primer	GTCGCGTTTCCTTTGCACTT
BST-2-Forward primer	TTAAGCGTGAGAATCGCGGA
BST-2-Reverse primer	CTAACCGTGTTGCCCCATGA
GAPDH-Forward primer	TGCACCACCAACTGCTTAGC
GAPDH-Reverse primer	GGCATGGACTGTGGTCATGAG