Supplementary Material

B-Cell Receptor-Associated Protein 31 Regulates the Expression of Valosin-Containing Protein Through Elf2

Cong-cong Jia[a] Juan Du[a] Xia Liu[a] Rui Jiang[a] Yongye Huang[a]
Tianyi Wang[a] Yue Hou[a] Bing Wang[a,b]

[a]College of Life and Health Science, Northeastern University, Shenyang, [b]Key Laboratory of Medical Image Computing, Liaoning Provine, Shenyang, China

Table S1 Sequence details of the primers used for the plasmid construction.

Name	Sense primers (5'---3')	Antisense primers (5'---3')
VCP overexpression primers	*ccgctcgag*atggcctctggagccgat	*cggatcc*ttagccatacaggtcatc
Elf2 overexpression primers	*ggatatc*atggcatcggcggtggttgac	*cggatcc*ttatttctcacacgtcactag
Bap31 overexpression primers	*ggatatc*atgagtttgcagtggactac	*cggatcc*tta**cttgtcatcgtcgtccttgta atcc**tcctcctcttcttgactga
Derlin1 overexpression primers	*ggatatc*atgtcggacatcggggact	*cggatcc*tcactggtctccaagtcg
VCP luciferase primers	*ggctagc*tggatttgagtgccttat	*gaagatct*gctcctgatccgcga
shRNA-Bap31	*caccg*ccatggcttatagatcattatcga aataatgatctataagccatgg	*aaaa*ccatggcttatagatcattatttcgata atgatctataagccatggc

Enzyme cleavage sites are shown in italics, and the sequences of flag are shown in bold.

Table S2 Gene primers used for the real-time PCR analysis as designed by Primer3 Input (version 0.4.0).

NCBI Reference	Name	Sense primers (5'---3')	Antisense primers (5'---3')
NM_001289726.1	*GAPDH*	aggtcggtgtgaacggatttg	tgtagaccatgtagttgaggtca
NM_001313698.1	*Bap31*	gccaccttcctctacgcag	tgccataggtcactaccaactc
NM_001109992.1	*PTPN11*	gggtcatgcgtgttaggaac	ccaggtccgaaagtggtact
NM_011102.4	*PRKCG*	actccacctttcagactccg	agatgatgccctggttgtga
NM_024236.2	*QDPR*	ctccccgttaccctggatac	ttcctgagtttggccgtttg
NM_178628.5	*ATL1*	ttgagagattcggccacagt	ttcttccattgccaacctgc
NM_145965.2	*GPHN*	ccccattccctgcatcagta	taacccgcatcacttgtcct
NM_001122818.2	*PNPLA6*	cgggtgcagaaaactccag	cgcataatcttccggccataga
NM_026688.2	*NDUFS3*	cagggatcacaccaatgcac	agagttgaaccgcagagaca
NM_029272.3	*NDUFS7*	tcctactcggttgttcgtgg	cctgcggtaccagatcttca
NM_133666.3	*NDUFV1*	atggatcgctcgacagacat	gtggccttctatctgcttgc
NM_020569.3	*PARK7*	atgcaaggtcacaacacacc	tgcaaactcaaagctggtcc
NM_010414.3	*HTT*	gtgccggggttgatattcac	actgggtacgttcggtgaat
NM_001038609.2	*MAPT*	actgagaacctgaagcacca	gtccaccggcttgtagacta
NM_008447.4	*KIF5A*	aagcttcaggccgactatga	ttgtggagggtctggagttc
NM_001131020.1	*GFAP*	gagggacaactttgcacagg	tcctccagcgattcaacctt
NM_010273.4	*GDI1*	tggaaaatggcaaggtggtg	attcttgatggggtggctga
NM_008810.2	*PDHA1*	aggacgaagaggaggttgtg	cattgtacttgcaggccagg
NM_001039509.1	*PNKD*	gagaggaagatgcagtgggt	aggcaatgggttctcaggaa
NM_011871.2	*PRKRA*	cagtctcccgaacacagact	atactgtccgttggcactca
NM_001136085.2	*UBA1*	tcgccgctgtccaagaaac	ctcgtctatgtctgcttcactg
NM_021284.6	*KRAS*	aaacttgtggtggttggagc	tgacctgctgtgtcgagaat
NM_008131.4	*GLUL*	caattgtgacccctatgcgg	gtattggaagggttcgtcgc
NM_009696.3	*APOE*	agctcccaagtcacacaaga	cccgtatctcctctgtgctc
NM_009976.3	*CST3*	agtacaacaagggcagcaac	acatgtagttcggcccatct
NM_026195.3	*ATIC*	cctggtgaaatgggaagcac	atgagccaagacaatcccca
NM_153064.4	*NDUFS2*	ccagtccctgcgaatcattg	ggaacttggtagccctctgt
NM_001160038.1	*NDUFS1*	caaagtagcagtgacgcctc	tagctgaggtggaacgagtg

Table S2 continued.

NCBI Reference	Name	Sense primers (5'---3')	Antisense primers (5'---3')
NM_001290469.1	*ATP1A3*	gagatcgtctttgcccgaac	gagtcattcacaccatcgcc
NM_015829.3	*SLC25A13*	atgtcgattttggaggcgtg	ctcaatccctgcgaatgtgg
NM_001048139.1	*BDNF*	ggcccaacgaagaaaaccat	gtttgcggcatccaggtaat
NM_001291059.1	*Elf2*	tggggcgagcgttaagatat	aagtcttcagggcaggtctc
NM_001163713.1	*TUFM*	atgagtgtgagttgctggga	accagagcacccaggttatc
NM_011400.3	*SLC2A1*	cagttcggctataacactggtg	gcccccgacagagaagatg
NM_010885.5	*NDUFA2*	cgttcacttatgccagcgtt	cattctgcatggctctggtt
NM_001159361.1	*DIP2B*	cagccacacatatcacctgc	gcccagcgtccttcttaaag
NM_008478.3	*L1CAM*	catacatggcagttgagggc	ggcagaaatagcgtccagtg
NM_021287.1	*SPTBN2*	ggatgggtgagcaggagttac	gcttgttccaatacctgatgctt
NM_016843.3	*ATXN10*	cttcaaggagcagcgtaacc	gcagactggaagccagatgtt
NM_019806.5	*VAPB*	atgttgtcaccaccaacctaaag	cctccatatcagaagtgtcaggc
NM_007435.2	*ABCD1*	aaatctaccctctagtacggcag	tttcccggcacaagactcg
NM_001290421.1	*FLNA*	gtggacaatgctgatggtactc	ctggccttcaccttactggc
NM_008292.4	*HSD17B4*	gactgtgttgccggaagatc	attcttgaatggtctgcggc
NM_145614.4	*DLAT*	agttaaagggacgggaccag	ttgctgatggggatgtctgt
NM_009503.4	*VCP*	cgagttcgcctaggagatgt	agagattgccagtgatgcct
NM_001079686.1	*SYNE1*	ctggctacagtgcgctaagg	tgagggcagacttggcatcta
NM_172809.3	*SACS*	tggagacctgacttctaaaacct	tggtggagttgtttgaccaaat
NM_145970.1	*CC2D1A*	taccaagctcctgagacagc	tcttctgccagcttctcgaa
NM_008991.2	*ABCD3*	caccttcacagcacacactc	ctagtcatttcacgcccagc
NM_173740.3	*MAOA*	agatccctgttgatgcacca	acactgcctcacataccaca
NM_010864.2	*MY05A*	gaggaagtgtggaaatcggc	tgagggctgtgaggtcattt

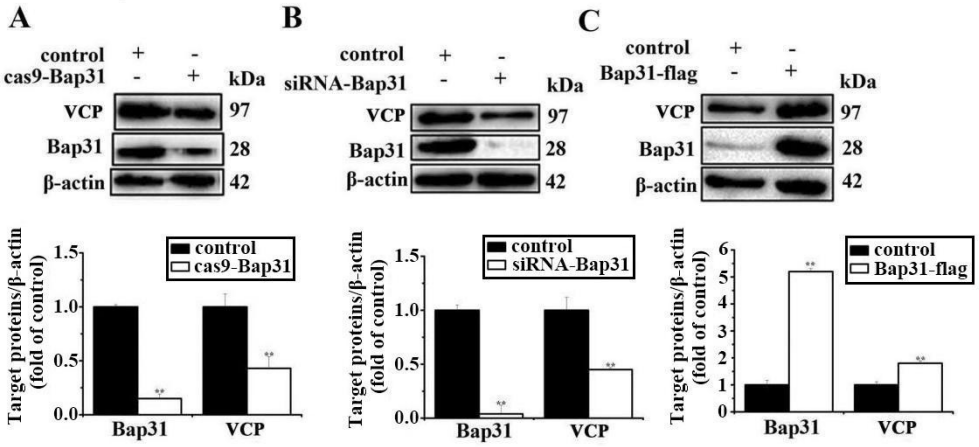

Fig. S1. Protein levels of VCP in N_2a cells and BHK cells. (**A**) N_2a cells were transfected with control vector or cas9-Bap31vector, after transfection for 72 hrs, the protein levels of VCP were detected by Western blot analyses in these groups. The protein levels of VCP in BHK cells transfected with siRNA-Bap31 (50 nM) for 72 hrs (**B**) and BHK cells overexpressing Bap31 for 72 hrs (**C**). Histograms showing the relative changes in the target protein levels in the Bap31 disturbed cells and control cells. p^{**} vs control < 0.01.

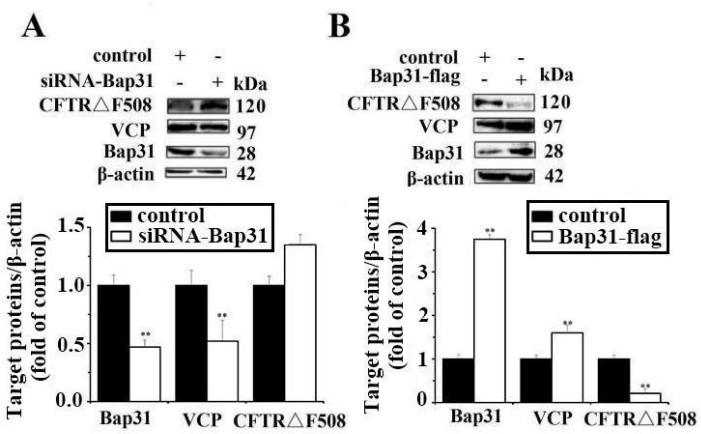

Fig. S2. Protein levels of VCP in cells stably expressing CFTRΔF508 with altered Bap31 expression. In BHK cells stably expressing CFTRΔF508-HA, anti-HA was used to detect the levels of CFTRΔF508. The protein levels of VCP were detected by Western blot analyses in CFTRΔF508-HA stable cells transfected with siRNA-Bap31(50 nM) for 48 hrs (**A**) and CFTRΔF508-HA stable cells transfected with Bap31-flag (2 μg) plasmids for 96 hrs (**B**). Histograms showing the relative changes in the target protein levels in the different groups. *p*** vs control < 0.01.

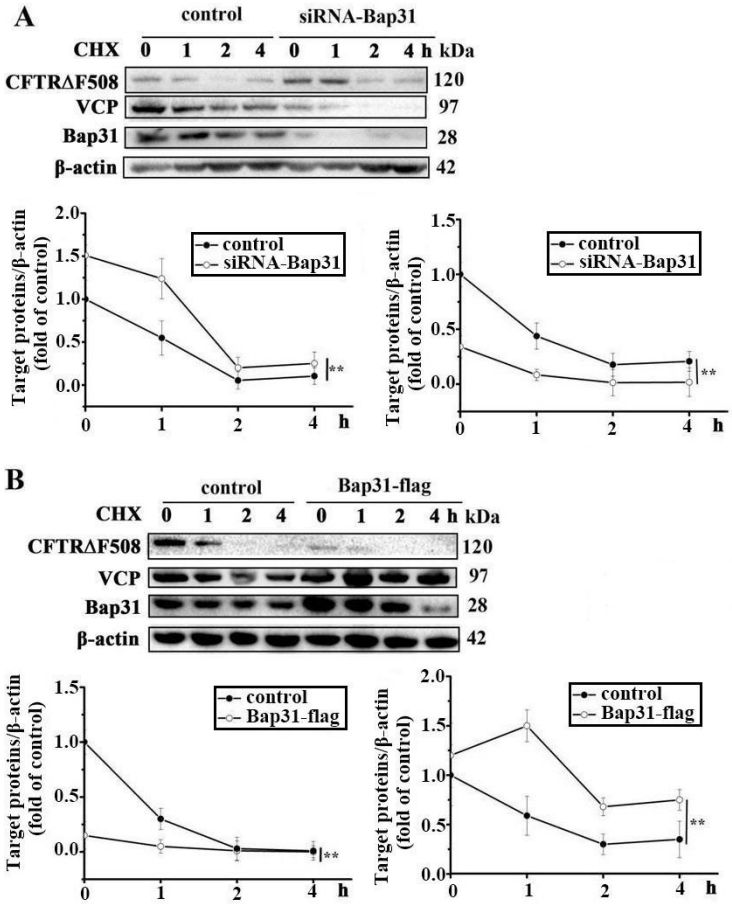

Fig. S3. The regulation of Bap31 on VCP in CFTRΔF508-HA stable cells. The protein levels of VCP and CFTRΔF508 were analyzed by Western blot analyses of CFTRΔF508-HA stable cells transfected with siRNA-Bap31 (**A**) or Bap31-flag plasmids (**B**) for 72 hrs, followed by CHX (250 μg/mL) treatment for 0, 1, 2 and 4 hrs. Cells were transfected with siRNA-negative or pcDNA3.1(-) as controls. Histograms showing the relative changes in the target protein levels in the different groups. *p*** vs control < 0.01.

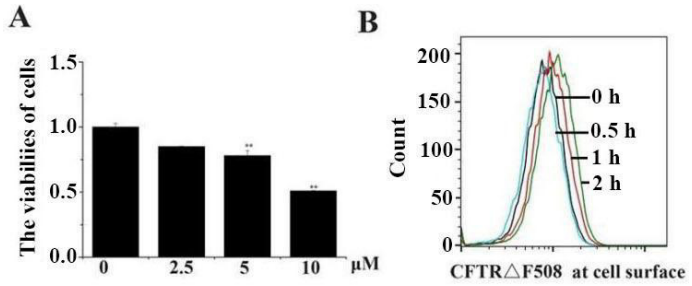

Fig. S4. Cell viabilities and the levels of CFTR△F508 at the cell surface. CFTRΔF508-HA stable cells were seeded in a 96-well plate and cultured with DBeQ (0, 2.5, 5 and 10 μM) for 2 hrs. Then, the absorbance was measured at 490 nm. Cell viabilities of these cells (**A**). CFTRΔF508-HA stable cells were cultured with 2.5 μM DBeQ (0, 0.5, 1 and 2 hrs), and the levels of CFTRΔF508 at the cell surface were analyzed by flow cytometry. *p*** vs control < 0.01.

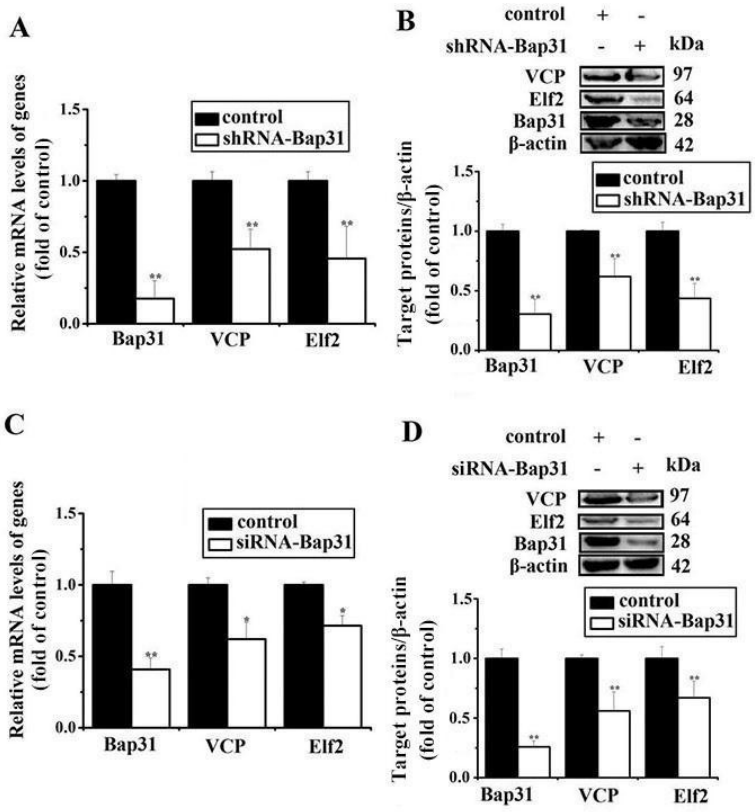

Fig. S5. The regulation of Bap31 on Elf2 in N_2a cells. Real-time PCR and Western blot analyses were used to detect the mRNA and protein levels of Elf2 in shRNA-Bap31 transfected cells (**A**, **B**) and cells transfected with siRNA-Bap31 for 72 hrs (**C**, **D**). Histograms showing the relative changes in the target protein levels in the different groups. *p** vs control < 0.05, *p*** vs control < 0.01.

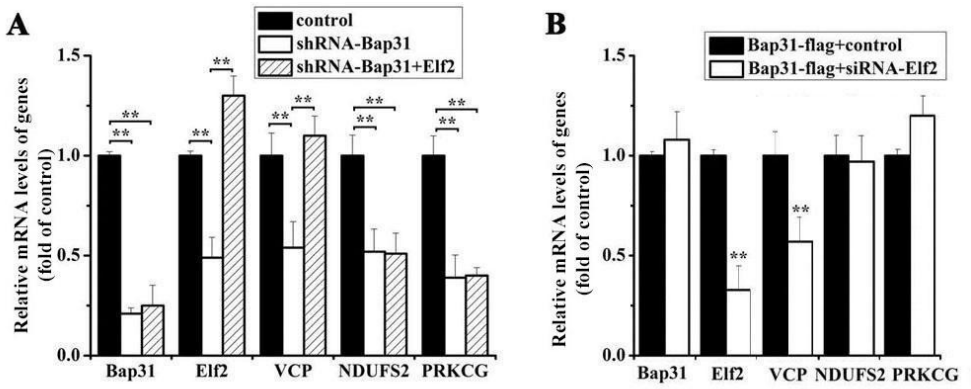

Fig. S6. The mRNA levels of genes in shRNA-Bap31 transfected cells overexpressing Elf2 for 72 hrs (A) and Bap31-flag cells transfected with siRNA-Elf2 for 72 hrs (B). *p*** vs control < 0.01.

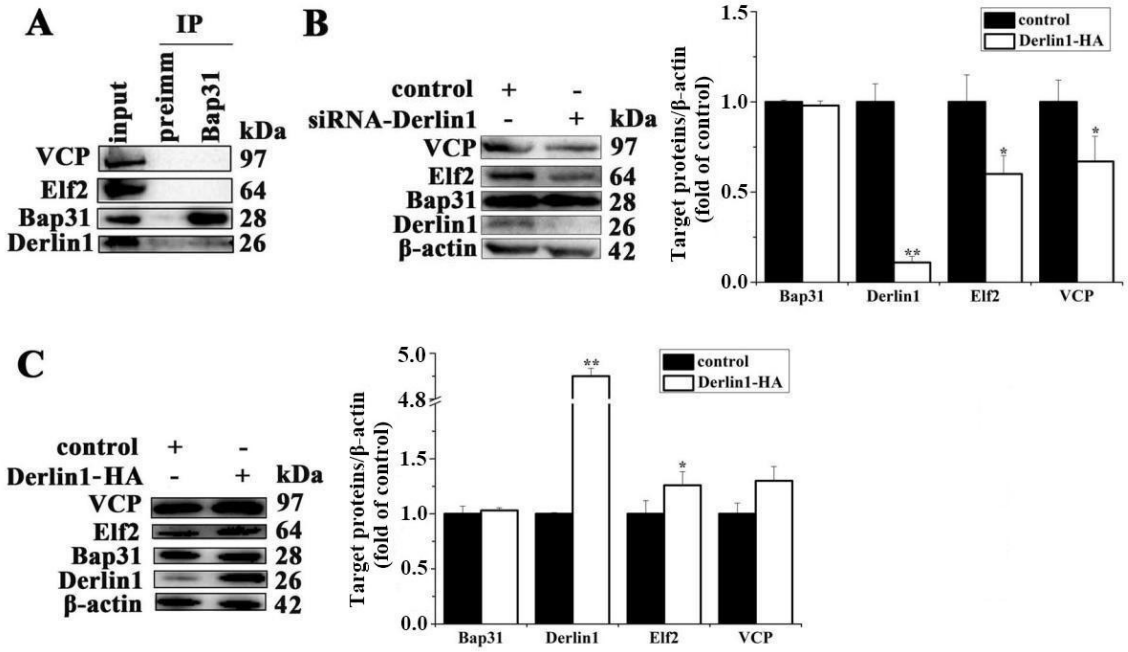

Fig. S7. The regulation of Bap31 on Elf2. Immunoprecipitation analysis of the interaction between Bap31 and Elf2 using Derlin1 as a positive control (A). Western blot analysis was used to detect the protein levels of Elf2 and VCP in cells transfected with siRNA-Derlin1 for 72 hrs (B) and cells transfected with Derlin1-HA plasmids for 72 hrs (C). Histograms showing the relative changes in the target protein levels in the different groups. *p** vs control < 0.05, *p*** vs control < 0.01.