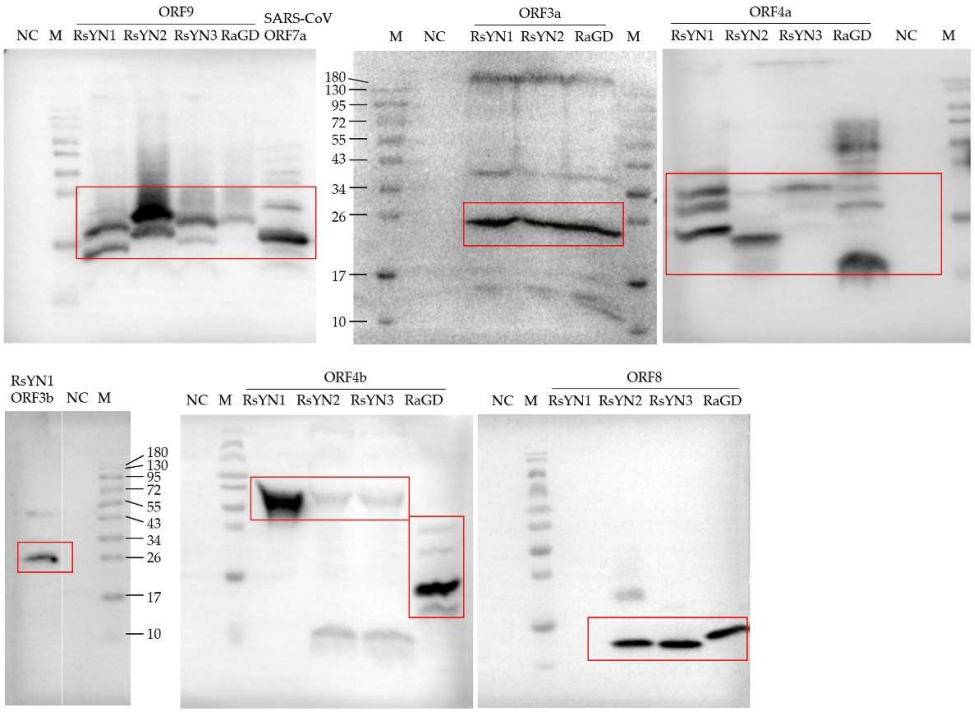
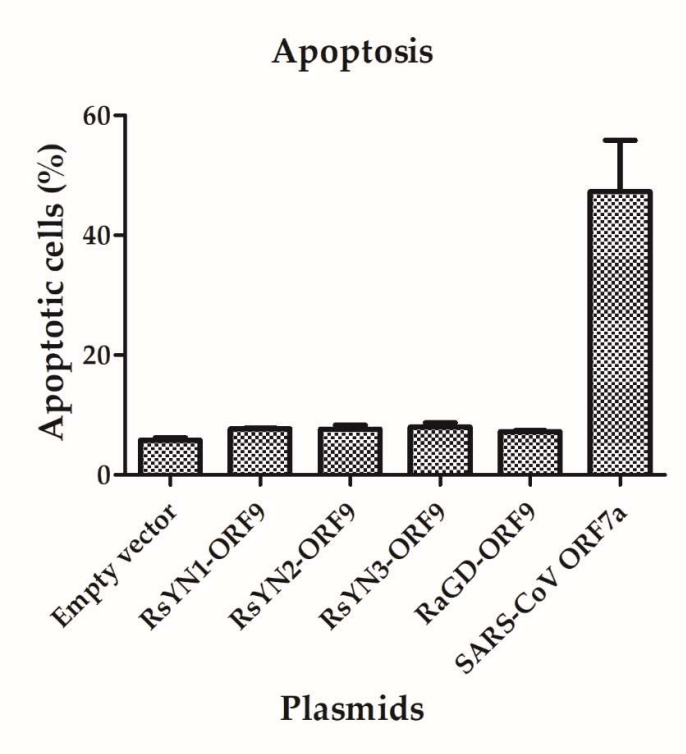
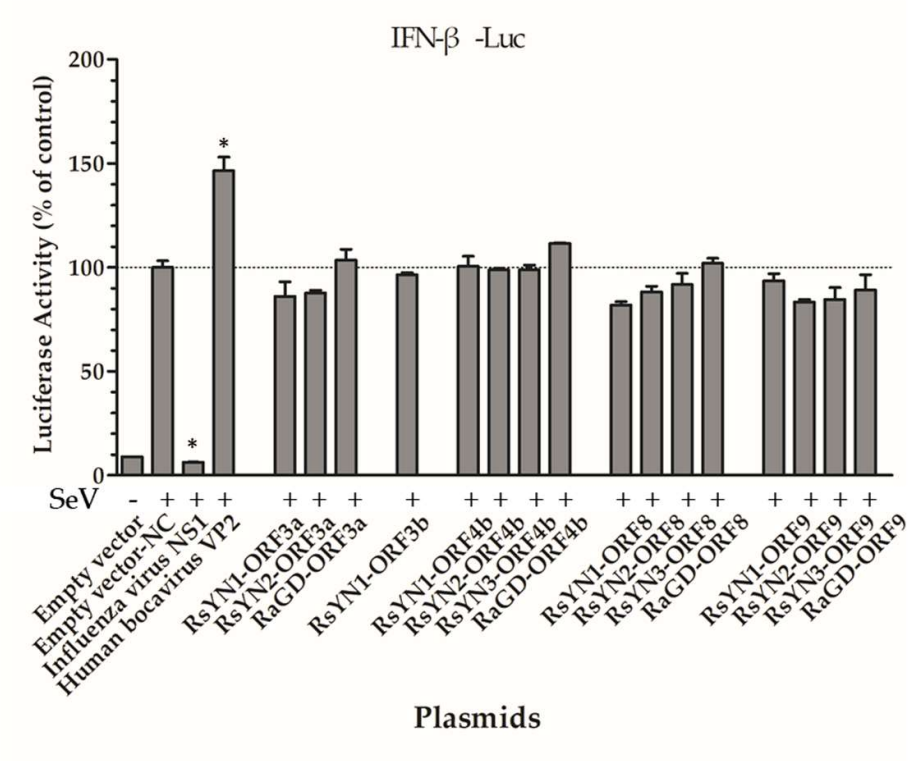


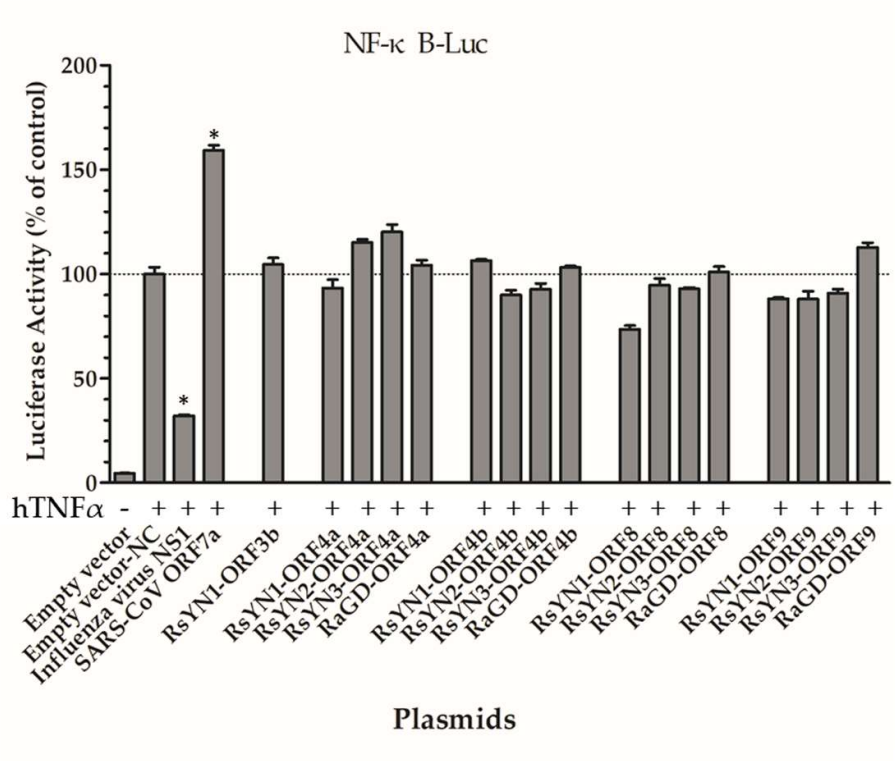
Supplementary Figure S1: western blot analysis the expression of accessory proteins. The HA-tagged proteins were detected with mAb against the HA tag. The bands circled in the red boxes indicate the expected proteins.



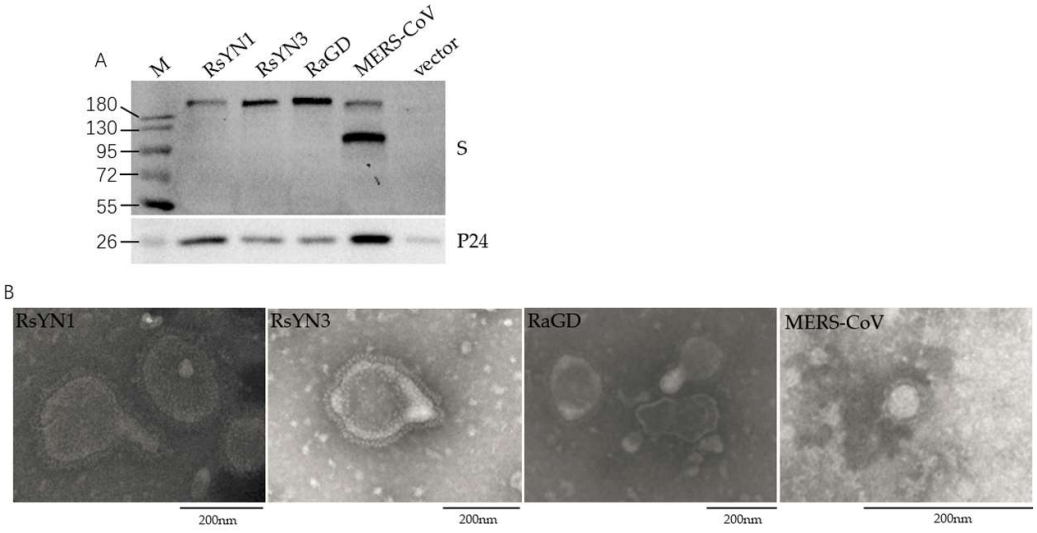
Sypplementary Figure S2. Apoptosis analysis of ORF9 proteins of BtCoV/Rh/YN2012. HEK293T cells were transfected with expression plasmids encoding ORF9 of different strains of BtCoV/Rh/YN2012, ORF7a of SARS-CoV Tor2, or pcAGGS vector for 24 h. Apoptosis was analyzed by flow cytometry after annexin V staining, and the percentage of apoptosis cells was calculated. Data are shown as the average from triplicate cell samples. Error bars indicate SDs.



Supplementary Figure S3. Functional analysis of ORF3a, ORF3b, ORF4b,ORF8 and ORF9 proteins on the production of Type I interferon. 293T cells seeded in 24-well plates were transfected with 100 ng pIFN-β-Luc, 5 ng pRL-TK, empty vector (625 ng), an influenza A NS1-expressing plasmid (625 ng), an human bocavirus VP2-espressing plasmid (625 ng), or accessory gene-expressing plasmids (625 ng). The cells were infected with Sendi virus (100 hemagglutinating units/ml) at 24 h posttransfection. Samples were collected at 6 h postinfection, followed by dual-luciferase assay. The results were expressed as the firefly luciferase value normalized to that of Renilla luciferase. Data are averages plus standard deviation for three experiments. Asterisks indicate significant differences between groups (compared with Empty vector-NC).



Supplementary Figure S4. Functional analysis of ORF3b, ORF4a, ORF4b,ORF8 and ORF9 proteins on the production of NF-κB. 293T cells were transfected with 100 ng pNF-κB-Luc, 10 ng pRL-TK, empty vector (500 ng), an NS1-expressing plasmid (500 ng), an SARS-CoV ORF7a-expressing plasmid (500 ng), or accessory genes-expressing plasmids (500 ng). After 24 h, the cells were treated with TNF-α. Dual-luciferase activity was determined after 6 h. The results were expressed as the firefly luciferase activity normalized to that of Renilla lucifetase. Data are averages plus standard deviation for three experiments. Asterisks indicate significant differences between groups (compared with Empty vector-NC).



Supplementary Figure S5. Characteristic of BtCoV/Rh/YN2012 spike mediated pseudovirus. (A) Western blot analysis the protein expression of pseudovirus in the medium supernatant. (B) Electron-microscopical observation of negative-stain coronavirus particles. The bars represent 200nm.

Table S1. General primers for AlphaCoVs genome sequencing

Primers	Primer sequence (5′-3′)
αCoV 5UTR 64 F1	CTMCTCAWYTCAACTAAACGA
αCoV 1 a770 R1	STCCAATCACCAACAGTCCA
αCoV 5UTR 275F2	TCTTGTYTGAAACCWGTAACTGT
αCoV 1a 674 R2	CCATTABYMAYAAAAGGAGAACCAA
aCoV 1a 12013 F1	ATGYTNDCHAATGGYTCTGG
aCoV 1b 448 R1	TCRTTYTCWACDGGRTCAWACCA
aCoV 1a 12076 F2	CARGAYWSTTATGGTGGTGCITC
aCoV 1b 202 R2	ATAHATGGAYTGCTCRTGBTCCAT
aCoV 1b 1810 F1	NATGGGHTGGGAYTAYCCIAA
aCoV 1b 3895 R1	ARRTCRTARTTMGTRCACATIGA
aCoV 1b 1999 F2	CCWGGTGGTACIACHTCIGGTGA
aCoV 1b 3613 R2	TGNGAYTTDCCACTACCAGGIGG
αCoV_18377_F1	GAAGGYDBYAATGGTGGTTC
αCoV_19745_R1	GGCATRGWRTAVCCRCAYTTCCA
αCoV_18425_F2	ACACCWGCWTWTGAYAARCGTGC
αCoV_19682_R2	YCYTTACACCAHARCATCCA
αCoV S23347 F1	BHTGTGYICAGTATTAYAAYGG
αCoV S25782 R1	GCMAGIACDARIGGCCAIA
αCoV S23367 F2	GHATHATGGTDYTICCIGGTGT
αCoV S24521 R2	ARCCAHACVYACCAIGGCCA

αCoV M271 F1	ACRCTYRTGCTGTGGATAATGTA
αCoV N950 R1	CNTYRCYACCAAACAGCA
αCoV M452 F2	TRGARGGCWWTAAGGTTGCTAC
αCoV N157 R2	TTGHTCADTCCARTAKCCAAT

The primers were designed targeting the conserved regions of the genomes. Nested PCR primers were designed in this experiment. Primer pairs F1/R1 for the first round PCR and F2/R2 for the second round PCR.

Table S1. Primers for the detection of viral sugbenomic mRNAs

Primers	Primer sequence (5′-3′)
RsYN1 sg F1	CATGGGGACTTAAAGTACATATCT
RsYN1 sg F2	AGAGTGTGTTCTTTCTAGACCTC
RsYN1 1ab 658R1	GCACTCCAACAGCATCATACA
RsYN1 1ab 542R2	GTGCCACACCAAGTGATAGC
RsYN1 sgS 694R1	GGAAGCCATTACAGTCATCACA
RsYN1 sgS 505R2	CAACATTAGAGGACCAGGTAACAC
RsYN1 sg3a 553R1	GCTCAACACGCCTAACTAAGG
RsYN1 sg3a 452R2	CGCACATAATGTTCTCCTCCAA
RsYN1 sg4a 307R1	GTGGTGTATTAGGTCTAACGATGT
RsYN1 sg4a 157R2	TAGGGTTGAAAGAATGACTACTCTG
RsYN1 sg4b 334R1	GGAATTGAAGTGGTGTGCTTAA
RsYN1 sg4b 286R2	CACTACTGAATGAGTACCATTGTTG
RsYN1 sgE 213R1	AACGGGACAGGGCGCAATT
RsYN1 sgE 140R2	TGGGACATAAGCCTGTGACA
RsYN1 sgM 540R1	AATGATAGTGGTAGTTGGCTTGG
RsYN1 sgM 441R2	ACTAAGAACAGTGAGCGTAATGC
RsYN1 sgN 626R1	GCCTGTGAATTGTTAGACTGGTT
RsYN1 sgN 501R2	ACCACTAGAAGCAGAGCGATT
RsYN1 sg8 97R1	AACTATTATAGAGCTGTCCAACGAC
RsYN1 sg8 80 R2	CCAACGACAAGCTCGAAGAA
RsYN1 sg9 285R1	TTGTGAGTAGCGAGTGTGAGT
RsYN1 sg9 258R2	GCTGACAGGTCTAACATGATAAGT
RsYN2 sg 2F	CATGGGGACTTAAAGTACATATCT
RsYN2 sg 36F	AGAGTGTGTTCTTTCTAGACCTC
RsYN2 sg1ab 589R	GTGAAGCAACAGCCGTAGTG
RsYN2 sg1ab 541R	TTCCACACCAAGTGATAGCATT
RsYN2 sgS 696R	CTCGCACACGCTATATTCCTTA
RsYN2 sgS 525R	AGTGACATAATTAGAGGACCAAGTG
RsYN2 sg3a 559R	GTTCAACTCGTCTAACAAGTGGTA
RsYN2 sg3a 435R	CATTACGATGTATGGTTGGTCTTC

RsYN2 sg3b 316R	CTAGAAGCAGTGGTATGTTAGGTT
RsYN2 sg3b 275R	ATGGACACGTAACCACTCTCA
RsYN2 sg3c 386R	GCAAGTAGCAGGAAGTAATAATGTC
RsYN2 sg3c 330R	GTTAGTAGTAGGGCTCTGTGTTG
RsYN2 sgE 224R	TAAACATCTAAGACGGGACAGG
RsYN2 sgE 151R	CGTAAACCGTGTTGGACATAAG
RsYN2 sgM 590R	CCTGTGTTGGAACGAGTGTT
RsYN2 sgM 447R	ACTTAGAACAGTGAGCGTGATG
RsYN2 sgN 637R	CCTGTTGGTTCTGTGGTTGAC
RsYN2 sgN 471R	ACTGCGATCACGGTTGGTA
RsYN2 sg7a 259R	AAGCCGAGCTATACAACTGTG
RsYN2sg7a 197R	ACTGGACAGAATATTGGTGGTAATG
RsYN2 sg7b 310R	GGCACTGCAACTCATTACCTT
RsYN2 sg7b 282R	GGAGTAGCGTGTATGTGTTCTG
RsYN3 sg 2F1	CATGGGGACTTAAAGTACATATCT
RsYN3 sg 36F2	AGAGTGTGTTCTTTCTAGACCTC
RsYN3 1ab 589R1	GTGAAGCAACAGCCGTAGTG
RsYN3 1ab 541R2	TTCCACACCAAGTGATAGCATT
RsYN3 sgS 665R1	CGTGCATTAGTTGTGATGTTGAG
RsYN3 sgS 514R2	TAGAAGACCAAGTAACGCCAAG
RsYN3 sg3a 559R1	GTTCAACTCGTCTAACAAGTGGTA
RsYN3 sg3a 435R2	CATTACGATGTATGGTTGGTCTTC
RsYN3 sg4a 316R1	CTAGAAGCAGTGGTATGTTAGGTT
RsYN3 sg4a 275R2	ATGGACACGTAACCACTCTCA
RsYN3 sg4b 386R1	GCAAGTAGCAGGAAGTAATAATGTC
RsYN3 sg4b 330R2	GTTAGTAGTAGGGCTGTGTGTTG
RsYN3 sgE 224R1	TAAACATCTAAGACGGGACAGG
RsYN3 sgE 151R2	CGTAAACCGTGTTGGACATAAG
RsYN3 sgM 590R1	CCTGTGTTGGAACGAGTGTT
RsYN3 sgM 447R2	ACTTAGAACAGTGAGCGTGATG
RsYN3 sgN 637R1	CCTGTTGGTTCTGTGGTTGAC
RsYN3 sgN 471R2	ACTGCGATCACGGTTGGTA
RsYN3 sg8 199R1	AAACTGGTAGGAACACTGGG
RsYN3 sg8 142R2	CTAAGTTAGCATCAAGCACCTT
RsYN3 sg9 344R1	AGCAGGATGAGAAGAATCACAAG
RsYN3 sg9 275R2	GAATAACGAGTGTGCGTCCTAA
RsGD sg 2F1	CATGGGGACTTAAAGATATTATCT
RsGD sg 40F2	GTGTCTTTCTTAGACCTCGTGTC
RsGD 1ab 491R1	GGAACATCAACACGGTCAACA
RsGD 1ab 463R2	CCTTAGTATATGTGGTGCCATTCA
RsGD sgS 530R1	GACCATGTAACACCTAAGACTTGA
RsGD sgS 455R2	CCTCTATTGTGGTTTGGTGATAAGT

RsGD sg3a 533R1	ACACATCTAGTAAGTGGTACATCC
RsGD sg3a 488R2	AACATCAGCAGCAACCACAA
RsGD sg4ab 412R1	CTAGCGGTAGCAGGTAATATAGTC
RsGDsg4ab 323R2	GGCTGTGTAATGGCTGTTGATT
RsGD sgE 168R1	AGCATTATGAACAGGCACAT
RsGD sgE 154R2	GCACATAAACTGTGTTGGACAT
RsGD sgM 575R1	GCGACTGTGATGTATGTAGGTAAT
RsGD sgM 489R2	TGTGAGCGTAATGCCAGTTG
RsGD sgN 480R1	CGAACGATTAGCAGACTGTGAA
RsGD sgN 339R2	GCCAAGTGAAGTAGGTTGAGTT
RsGD sg8 245R1	AGCAAACCAGCAATCAAGTCTA
RsGD sg8 193R2	GCCAGAAGACAGGTAGAAGTAC
RsGD sg9 357R1	CCAGAGGCAAACATAAAGGACTAA
RsGD sg9 283R2	GTGTGACTCTGACATTAGTTCTG

The forward primers were designed targeting the leader sequence at the 5′-end of the complete genome. The reverse primers were designed within ORFs of corresponding gene. Nested PCR primers were designed in this experiment.

Table S3. Different cell line susceptibility to BtCoV/Rh/YN2012 RsYN1-, RsYN3-, RaGD- and MERS-CoV-spike mediated pseudovirus.

Cell lines	**RsYN1**	**RsYN3**	**RaGD**	**MERS-CoV**
Human				
Respiratory tract				
Hep-2	-	-	-	-
A549	-	-	-	+/-
Calu-3	-	-	-	++
H292	-	-	-	-
Intestinal tract				
Caco-2	-	-	-	++++
Liver				
Huh-7	+/-	-	-	++++
Genitourinary tract				
Hela	-	-	-	-
HEK-293T	-	-	-	+/-
Muscle cell				
RD	-	-	-	-
Nerve cell				
CCF-STTG1	-	-	-	-
Immune cell				
THP-1	-	-	-	-
Bats				
RsKT	-	-	-	+
RsLu4323	-	-	-	++
RsBrT	-	-	-	-

RlKT	-	-	-	-
HpLuT	-	-	-	-
Tb1-Lu	-	-	-	-
RaK4324	-	-	-	-
Paki	-	-	-	-
Other mammals				
LLC-MK2	-	-	-	++
Vero	-	-	-	++
BHK21	-	-	-	-
MDCK	-	-	-	-
FK	-	-	-	-
PK15	-	-	-	-
SIEC	-	-	-	-
NIH-3T3	-	-	-	-
V79	-	-	-	-

Note: The luciferase signal intensity was normalized to fold changes compared to mock. "-", <2-fold increase; "+/-", 2-5 fold increase; "+"5-20 fold increase, "++" 20-100 fold increase, "++++", >1000 fold increase.