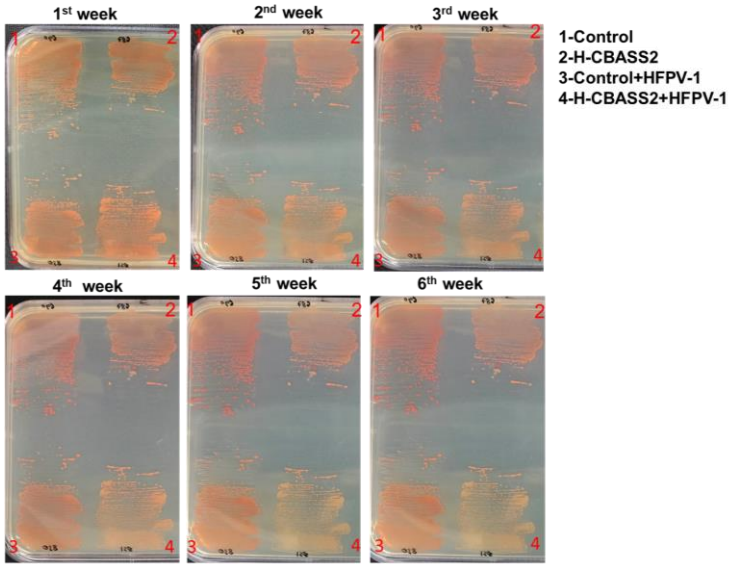
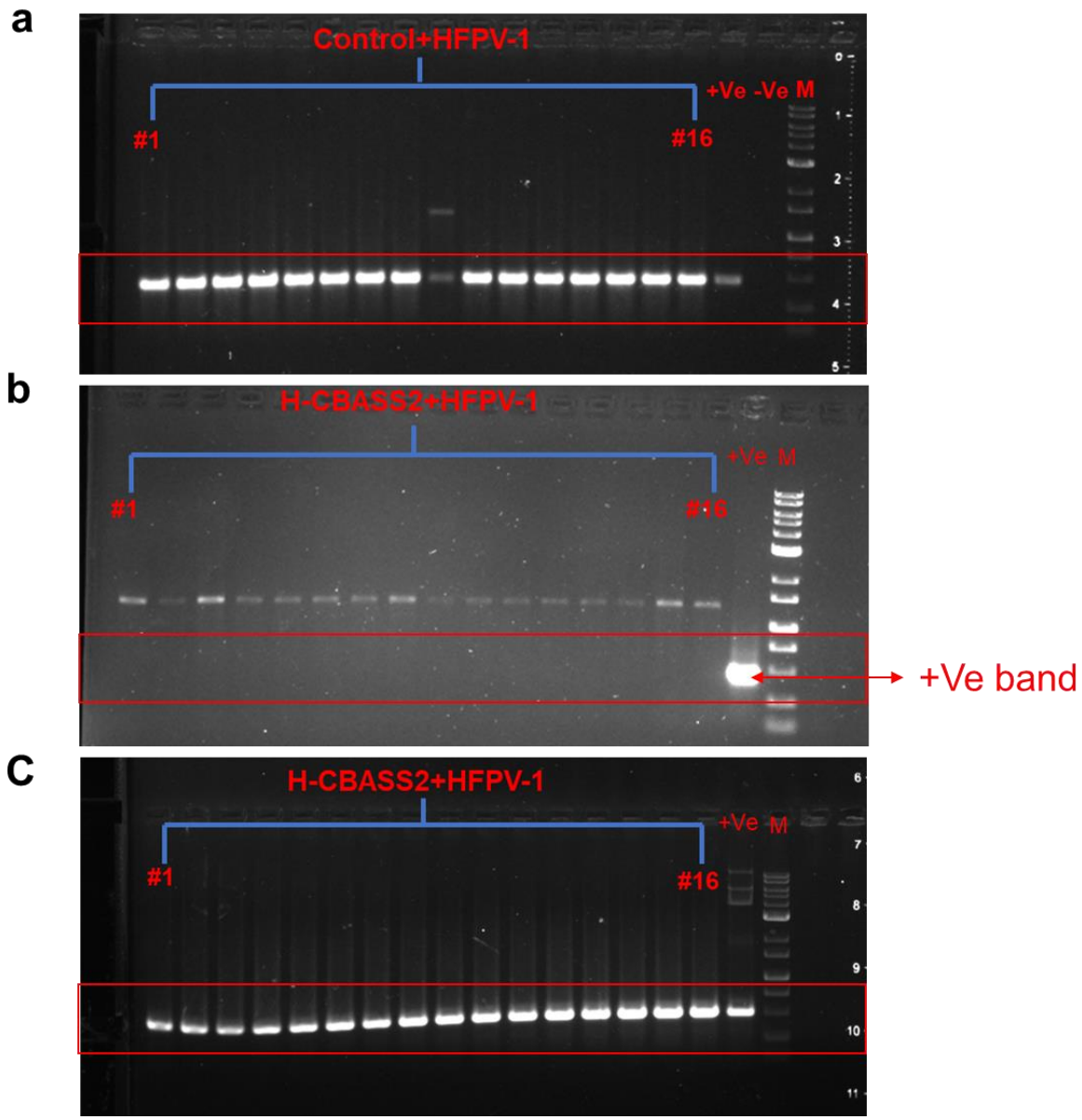
```
Query  3    ELPSKFEEFLSNIRPTDKQISDYVDGHEQLRERLSNDGDLSEIHVSDFLQGSYARRTAVK  62
            EL  +F EFL+NIRPTD Q  D+  G   LRERL N   L EI VS FLQGS  R TA++
Sbjct  3    ELQPQFNEFLANIRPTDTQKEDWKSGARTLRERLKNFEPLKEIVVSTFLQGSIRRSTAIR  62

Query  63   PIGDEKSDVDIVFVTNLPKSEYTASDAMELCEPFLNRYYPGQWEPNQRSYKIELNKVEMD  122
            P+GD++ DVDIV VTNL  +  + +DAM+L  PFL +YYPG+WE   RS+ I L+ VE+D
Sbjct  63   PLGDKRPDVDIVVVTNLDHTRMSPTDAMDLFIPFLEKYYPGKWETQGRSFGITLSYVELD  122

Query  123  LVLTAAPSEAV----INELSAPGSIGKANIANIADQQDMGIIAKSIDNTFEGAEKN----  174
            LV+TA P        + +L    S+   N  ++ +Q D  +      NT   +E N
Sbjct  123  LVITAIPESGAEKSHLEQLYKSESVLTVN--SLEEQTDWRLNKSWTPNTGWLSESNSAQV  180

Query  175  -------WQDEPLDIPDRELDSWDQTHPLATLDWTQNKNDRTNGHYINVVKALKWWRRTQ  227
                  W+  PL +PDRE + W +THPLA + WT  KN   NGHYIN+V+A+KWWR+
Sbjct  181  EDAPASEWKAHPLVLPDREKNEWGRTHPLAQIRWTAEKNRLCNGHYINLVRAVKWWRQQN  240

Query  228  V-DFPERPKSYPLERLIGECCPDYISSVAEGVVRAFDVFIEKYESNAEHEDTPVLGQHGI  286
              D P+ PK YPLE LIG    +  +S+A+G+V+  D F+ ++ +    +  P L  HG+
Sbjct  241  SEDLPKYPKGYPLEHLIGNALDNGTTSMAQGLVQLMDTFLSRWAAIYNQKSKPWLSDHGV  300

Query  287  PENNVLARLEGRDFAAFYGRVTDASEVAQRAYEEEDKEQSALYWREMFGEEFPLIGDSDD  346
             E++V+ARL   DF +FY  +  A+E+A+ A   E+ ++SA  WR++FG +FPL G
Sbjct  301  AEHDVMARLTAEDFCSFYEGIASAAEIARNALASEEPQESAQLWRQLFGSKFPLPGPQGG  360

Query  347  DDTDDESTATFTPPNNTA  364
            D         FT P+  A
Sbjct  361  D-----RNGGFTTPSKPA  373
```

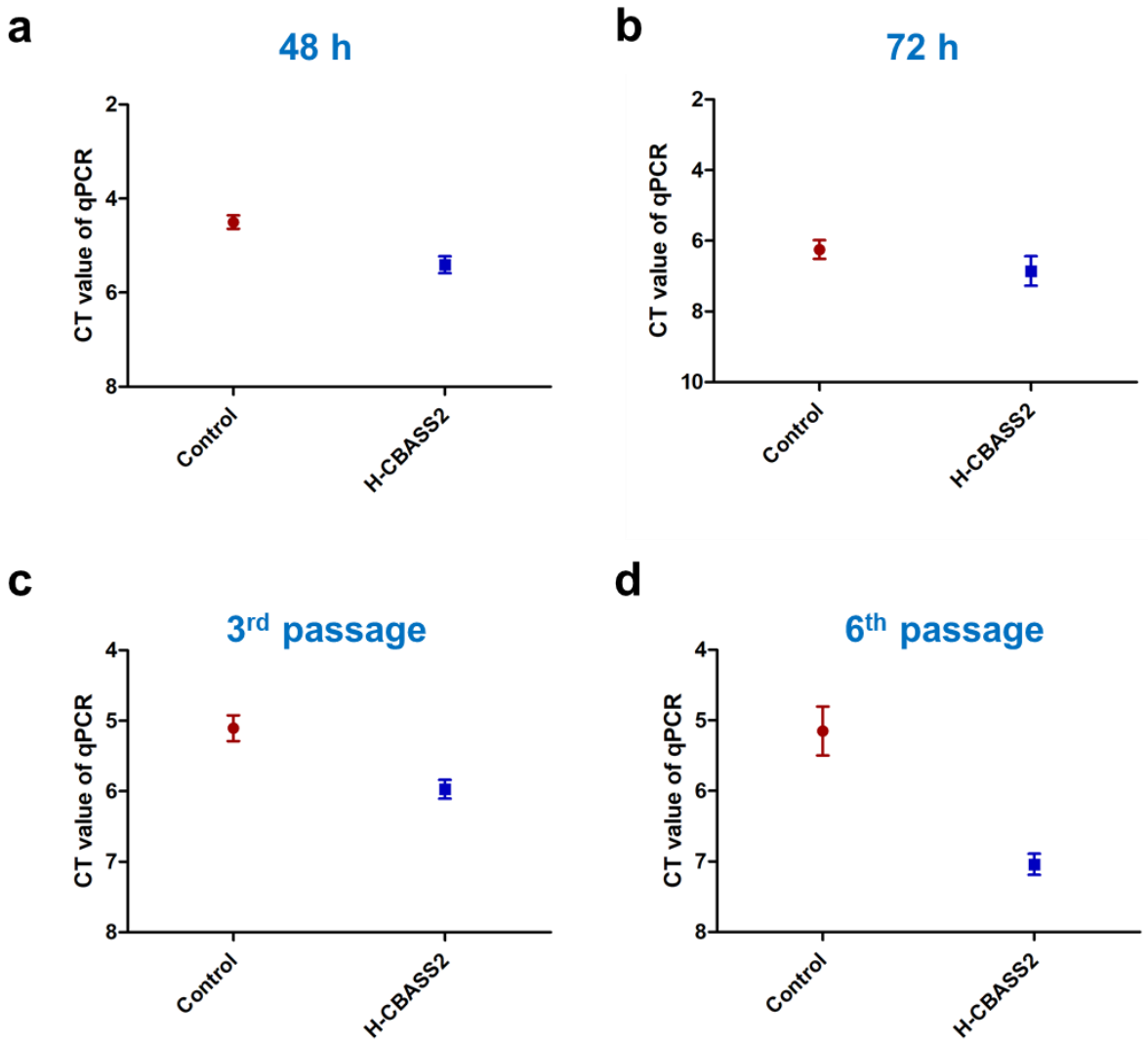
Extended Fig.1 A pairwise BLASTP alignment of the H-CBASS2 cyclase with the *E. cloacae* cyclase (CdnD). The catalytically important residue, highlighted in yellow, has been mutated to render the cyclase inactive.



Extended Fig.2 Representative images showing a gradual bleaching of the virus-infected H-CBASS2-expressing colonies. A time series analysis was conducted on colony color with or without viral infection in the H-CBASS2-expressing strain, as well as in the infected control containing an empty vector.



Extended Fig.3 Most H-CBASS2-expressing colonies are virus-negative after passage 10. The results of ethidium bromide-stained agarose gel electrophoresis of products from colony PCR. a) Virus detection via PCR after the 10th passage in virus-infected *H. volcanii* strains containing an empty vector (control). The gel displays colonies #1 to 16 from left to right after the 10th passage, along with positive control, negative control, and a 1Kb DNA ladder (NEB). The expected band size is ~500 bp. b) Virus detection via PCR after the 10th passage in virus-infected *H. volcanii* strains expressing H-CBASS2. The gel displays colonies #1 to 16 from left to right, with a positive control and a 1Kb DNA ladder (NEB). The expected band size is ~500 bp. c) H-CBASS2 detection via PCR by amplifying an internal region after the 10th passage in virus-infected *H. volcanii* strains expressing H-CBASS2. The gel shows colonies #1 to 16 from left to right, with a positive control and a 1Kb DNA ladder (NEB). The expected band size is ~550 bp.



Extended Fig.4- CBASS-expressing cells exhibit a reduction in viral DNA levels. qPCR was performed to quantify the amount of viral DNA in the supernatant of HFPV-1 infected *H. volcanii* cells after 48 and 72 hours of growth, as well as after the 3rd and 6th passages, using specific primers targeting the HFPV-1 genome. **a**) Ct values for free viral DNA in the supernatant after 48 hours. **b**) Ct values for free viral DNA in the supernatant after 72 hours. **c**) Ct values for free viral DNA in the supernatant after the 3rd passage. **d**) Ct values for free viral DNA in the supernatant after the 6th passage.