

# No Substantial Histopathologic Changes in *Mops condylurus* Bats Naturally Infected with Bombali Virus, Kenya

## Appendix

### IHC protocol

Antigen retrieval was performed in citrate buffer (pH 6). Tissue sections were prepared on slides and incubated for 20 min at a maximum temperature  $>99^{\circ}\text{C}$  during antigen retrieval. After antigen retrieval, the sections were washed with  $1\times$  Tris-buffered saline (TBS) for 5 min and then incubated in 3% hydrogen peroxide for 10 min. Next, the sections were washed  $2\times 5$  min each in  $1\times$  TBS and then incubated in 10% bovine serum albumin for 20 min. Rabbit polyclonal antiserum Ag204 against Ebola virus matrix protein VP40 (11, main text) was diluted 1:500 in Animal Free Blocker and Diluent (Vector Laboratories, <https://www.vectorlabs.com>) and added to the tissue sections. Sections were incubated for 60 min with the primary antibody and then washed  $2\times 5$  min each in  $1\times$  TBS. For each sample, a negative control was similarly prepared; the primary antibody was replaced with a rabbit isotype control diluted at 1:500. Secondary antibody (BrightVision anti-rabbit HRP 1 step detection system) (Immunologic, <http://www.immunologic.nl>) was added to the sections and incubated for 30 min. The sections were washed  $2\times 5$  min each in  $1\times$  TBS. Bright DAB chromogen (Immunologic) was added to the sections and incubated for 6 min; the slides were then rinsed in water. Sections were counterstained with hematoxylin (Papanicolaou's solution 1a Harris hematoxylin solution, Sigma-Aldrich, <https://www.sigmaaldrich.com>) for 10 s and rinsed with tap water for 3 min.

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CLUSTAL O(1.2.4) multiple sequence alignment

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      MRRVILPTAPPEYMEAIYPARNSSTIARGGNSNTGFLTPEVNGDTPSNPLRPIADDTID      60
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      MRRTVIPTAPPDYTEALYPQRANSIGSTSTSTNQYYPDVPGGDIPSNLSRPVADDNID      60
*:*.:*****:* **:* *:* * : . .:* : : ** ** **:*:*:*

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      HASHTPGSVSSAFIEAMVNVISGPKVLMKQIPIWLPLGVADQKTYSFDSSTAAIMLASV      120
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      HTNHTPDNVSSAFIEAMVNVISGPKVLMKQIPIWLPLGVADQKQYFDSSTAAIMLASV      120
*:*:*:*.....*****.....

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      TITHFGKATNPLVRVNLGPGIPDHLRLLRIGNQAFLQEFVLPVQLPQYFTFDLTALK      180
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      TITHFGKTSNPLVRVNLGPGIPDHLRLLRIGNQAFLQEFVLPVQLPQYFTFDLTALK      180
*****.:*****.....

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      LITQPLPAATWTDPTPTGSNGALRPGXSHPKLRPILLPNKSGKKGNSADLTSPEKIQAI      240
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      LITQPLPAATWTDDEVLLTSPNMLRPGLSFHPKLRPILLPGKPGKKGANLGLTAPDKIHAI      240
*****.:* . ***** * ***** * ***** . ** . **:*:*

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      MTSLQDFKIVPIDPTKNIMGIEVPETLVHKLTKKVTSKNGQPIIPVLLPKYIGLDPVAP      300
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      MDHLQDLKVVPIDSSKNIVGIEVPDALVQKLTGKKPHAKNGQSIIPVLLPKYIGDPVSA      300
* **.*:*:* :*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

tr|A0A0D5W8L9|A0A0D5W8L9_9MONO      GDLTMVITQDCDTCSPASLPVVEK      326
tr|A0A4D5SGV0|A0A4D5SGV0_9MONO      SDLTMVITQDCDTCNSPASFPIASEK      326
.******.*:*:*.* **

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**Appendix Figure.** Alignment of Ebola virus (UniProt no. A0A0D5W8L9) and Bombali ebolavirus (UniProt no. A0A4D5SGV0) VP40 matrix proteins. We used the UniProt protein database (<https://www.uniprot.org>) align-tool and found  $\approx 75\%$  amino acid identity between the 2 VP40 proteins. Asterisks represent fully conserved residues between sequences, colons represent conservation between amino acids with strongly similar properties, and periods represent conservation between amino acids with weakly similar properties.