

```

HMPREF1072_01167 - BSAP-2 ---LSANVSFDKESSNKTSITTKETYIRVDQIKQLERYQLYADPTVLADYLTPYFKSRLS 200
Bf638R_1646 - BSAP-1  SGLSSSSFSSTQHYTSKYSIADYTSFI-----RRRRLFLTASTELLISKYLTSMFVDDLS 174
                        **:.:*  :. :.* **:  ::*  :.* * * . :*:.*** * . **

BSAP-2  TASPDAIIEEFGTHILIDFNVGGRLSIFYKSTITDNLKVESKTKIAKGGITGAIKTVNLS 260
BSAP-1  KQSPSFIQHYGTHVITDITLGGRIITVLYRSSINTSKKTATV--EAGCASGIKMFNLS 231
                        .**.**:**:* *:*:* **:::*:* * . * . : * : * : .***

BSAP-2  FSGSSTTEVEQYQRRNSNWSCNVNMYGGQHDGH-TITITSDGATNHTFNLGSGWQQSVDK 319
BSAP-1  VDGHYDQTLVKDENSEE---IVYRTEGGDPSRALIGQLNYDSKNPSVIDISSWQQSCDD 287
                        ..*  * *:: :.:.  . **:.  . : . * . . :.:.*** **

BSAP-2  THCVLTEINFNKTYPIYEFIKDPIKKQQIKDAAEKY 355
BSAP-1  NNMTLVDAEPGLIPIYDLVSDMGKKEQLKLAVERY 323
                        ..*.: : . ***:.* **:* * .:*

```

Figure S1. Clustal-W alignment of the MACPF domains of BSAP-1 and BSAP-2

Amino acid sequence alignments of the MACPF domain of BSAP-2 (HMPREF1072_01167) and BSAP-1 (BF638R_1646) by Clustal-Omega (1). A conserved amino acid motif (Y/W-G-T/S-H-F/Y-X6-GG) of MACPF domains is boxed, including two glycine residues highlighted in yellow required for extension of the transmembrane beta hairpin (2). Residues of BSAP-1 previously shown by site-directed mutagenesis to be essential for functional activity (3) are highlighted in pink.

1. **Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG.** 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* **7**:539.
2. **Dunstone MA, Tweten RK.** 2012. Packing a punch: the mechanism of pore formation by cholesterol dependent cytolysins and membrane attack complex/perforin-like proteins. *Curr Opin Struct Biol* **22**:342-349.
3. **Chatzidaki-Livanis M, Coyne MJ, Comstock LE.** 2014. An antimicrobial protein of the gut symbiont *Bacteroides fragilis* with a MACPF domain of host immune proteins. *Mol Microbiol* **94**:1361-1374.