

(A)

| | | | | |
|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| 1 | 11 | 21 | 31 | 41 |
| M I I N H N L S A V | N A H R S L K F N E | L A V D K T M K A L | S S G M R I N S A A | D D A S G L A V S E |
| eebeebbebb f s f f f | ebbeebbeee s f f f | eeeebbeebe f s | eebeebbebe f f f s f s | eebebbbebe f f s f f s |
| 51 | 61 | 71 | 81 | 91 |
| K L R T Q V N G L R | Q A E R N T E D G M | S F I Q T A E G F L | E Q T S N I I Q R I | R V L A I Q T S N G |
| ebbebebebe f f f | ebbeebbebb s f f f | bbbebbbebb s fs ff | eebeebbeeb ff | ebbbebebe ff s f f |
| 101 | 111 | 121 | 131 | 141 |
| I Y S N E D R Q L V | Q V E V S A L V D E | V D R I A S Q A E F | N K F K L F E G Q F | A R G S R V A S M W |
| eeeeeeeeeb f f f | eeebebebe f f | beebbebebe f f | eeeebebebe f | eeeeeeeebe eeeeeeeebe |
| 151 | 161 | 171 | 181 | 191 |
| F H M G P N Q N Q R | E R F Y I G T M T S | K A L K L V K A D G | R P I A I S S P G E | A N D V I G L A D A |
| bebbbebebe f s | bebebebebe f | eebebebebe f | eebebebebe f | beebbebebe f |
| 201 | 211 | 221 | 231 | 241 |
| A L T K I M K Q R A | D M G A Y Y N R L E | Y T A K G L M G A Y | E N M Q A S E S R I | R D A D M A E E V V |
| bbbebebeeb f | ebbebebebe ff f ff | ebbebebebe f | eebebebebe f f f fs | eebebebebe f f s f |
| 251 | 261 | 271 | 281 | |
| S L T T K Q I L V Q | S G T A M L A Q A N | M K P N S V L K L L | Q Q I | |
| ebbeebbebe ss f | bbbebbbebe f fs f | eeeebbeb f | eee ss f f | |

(B)

| | | | | |
|-----------------------------|------------------------------|------------------------------|----------------------------|----------------------------|
| 1 | 11 | 21 | 31 | 41 |
| R I N S A A D D A S | G I A V S E K L R T | Q V N G I L R Q A E R | N T E D G M S F I Q | T A E G F L E Q T S |
| eebebebebe fff f f f s f | ebbbbebebe fs ss | ebbebebebe f f s | eebebbbebe f f s f | ebbebebebe ss f f f |
| 51 | 61 | 71 | 81 | 91 |
| N I I Q R I R V I A | I Q T S N G I Y S N | E D R O L V Q V E V | S A I V D E V D R I | A S Q A E F N K F K |
| ebbebebebe f f f f fs | bebebebebe f f f f | eebebebebe f f f f | eebebebebe s f f f | bebebebebe f f f f |
| 101 | 111 | 121 | 131 | 141 |
| L F E G Q F A R G S | R V A S M W F H M G | P N Q N Q R R E F C | I G T M T S K A L K | L V K A D G R P I A |
| bebebebebe f s | eebebebebe f | eebebebebe f | eebebebebe f f f | eebebebebe f |
| 151 | 161 | 171 | 181 | 191 |
| I S S P G E A N D V | I G I L A D A A L T K | I M K Q R A D M G A | Y Y N R L E Y T A K | G L M G A Y E N M Q |
| beeeeebeeb beeb | bebebebebe beeb | beeeeebeeb f f f | bebebebebe f f f | bebebebebe f f f |
| 201 | 211 | | | |
| A S E S R I R D A D | M A E E V V S | | | |
| eebebebebe f f f f s f f | bbbebebebe s f f f f | | | |

(C)

1 ↓

| | | | | |
|--------------------------------------|------------------------------------|----------------------------------|------------------------------------|----------------------------------|
| ADGASGLAVS | EKLRTQVNGL | RQAERNTEDG | MSFIFTQTAEGF | LEQTSNIIQR |
| e e e e e b b b b f f f f f s s s | e b e e b e e b f f f f f f f | e b e e b e e b s f f f f f | b b b b e b e e b s f s s f f f | b e b e b b e e f f f f f f f |
| 51 | 61 | 71 | 81 | 91 ↓ |
| IRVLAIAQTSN | GIYSNEDRQL | VQVEVSALVD | EVDRRIASQAE | FNEFKLFEGQ |
| b e e b b b b e e f f s f f f f f | e e e e e e e e f f f f f f f f | b e e b e e b f f f f f f f | e b e e b b e e f f s f f f f f | e e e e b b e e f f f f f f f |
| 101 | 111 | 121 ↓ | 131 | 141 |
| FARGSRVAVSM | WFHMGPQNQ | RERFYVTGTMT | SKALKLVKAD | GRPIAISSPG |
| e e e e e e e e f s | e b e b e e e e e b e b e e e e | e b e b e e b e b e b e e b | b e e b e e e e e b e b e e e e | e e b e b e e e e |
| 151 | 161 | 171 | 181 | 191 |
| EANDVIGLAD | AALTAKIMKQR | ADMGAYYNNI | EYTAKGIMGA | YENMQASESR |
| e b e b b b e e f | e b b e e e e f f f f f f f | b e b e b e e b f f f f f f f | e b b e b e e b f f f f f f f | b e b e b e b e f f f f f s f |
| 201 | 211 | | | |
| IRDADDMAEEV | VSLT | | | |
| b e b e b b e e s f f f s f f f | e e e e f | | | |

(D)

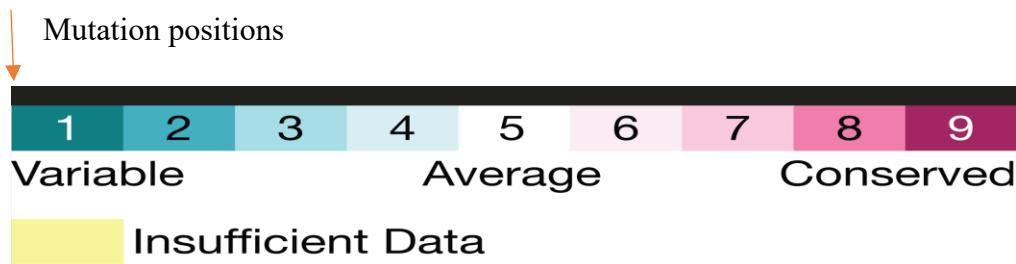
| | | | | |
|--------------------------------------|------------------------------------|------------------------------------|--------------------------------------|--------------------------------------|
| 1 | 11 | 21 | 31 | 41 |
| SAADDASGLA | VSEKLRTQVN | GLRQAERNTE | DGMMSFIQTAE | GFLEQTSNII |
| e e e e e b b b b f f f f f s s s | b b e b e e b e s s f f f f f | e b e b e e b e f f s f f f f | e b b b b e b b e f f s f s f s f | e b b e b b e b b f f f f f f f f |
| 51 | 61 | 71 | 81 | 91 |
| QRIRVLAIAQT | SNGIYSNEDR | QLVQVEVSAL | VDEVDRRIASQ | AEFNKFKLFE |
| e e b e b b b e b f f f f s f f f | e e e e e e e e f f f f f f f f | e e b e b e e b f f f f f f f f | e e b e b e e b f f f f f f f f | e e e e e b b e f f f f f f f f |
| 101 | 111 | 121 | 131 ↓ | 141 |
| GQFARGSRVA | SMWFHMGPQNQ | NQRERFYVIGT | MTSKALKSVK | ADGRPIAISS |
| e e e e e e e e f s | e b e b b b e e e e b e b e e e | e e b e b e b e e e b e b e b e | b e b e b e e e e b e b e b e e | e e e e b b e e e b e b e b e e |
| 151 | 161 | 171 | 181 | 191 |
| PGEANDVIGL | ADAALTAKIMK | QRADMGAYYNN | RLEYTAKGLM | GAYENMQASE |
| e e b e b b e e b e b b e e e e | b e b b e e e e e e b e b e e e | e e b e b e b e f f f f f f f f | e b e b e b e e f f f f f f f f | e b b e b e b e f f f f f f f f |
| 201 | 211 | | | |
| SRIRDADMAE | EVVSLT | | | |
| b e b e b b e e s f s f f f f | e e e e e f f f f f | | | |

Additional File 3: Figure S1: FlaB protein of *L. interrogans* (A) (WP_000586170, template), variant protein sequences of NHSL13SL215 (B) (WKR44734) NHSL13ML092 (C) (WKR38874), and NHSL13ML506 (D) (WKR38873). Conserved regions of *L. interrogans* FlaB protein sequence and partial FlaB variant protein sequences

of the patient sample created by the ConSurf web server (https://consurf.tau.ac.il/consurf_index.php).

Amino acid sequence (WKR38874) deduced from the nucleotide sequence of patient number, NHSL13ML092 (WKR38874). The variant D3G (D42G in wild type) is located in a highly conserved region. The other two variants K93E and I126T (K132E and I165T in template) are located in variable regions.

Amino acid sequence (WKR44734 and WKR38873) deduced from the nucleotide sequence of patient number, NH SL13SL215 and NHSL13ML506. The mutation Y130C (Y164C in wild type) and L138S (L175S in wild type) are located in variable regions.



- e** - An exposed residue according to the neural network algorithm.
- b** - A buried residue according to the neural network algorithm.
- f** - A predicted functional residue (highly conserved and exposed).
- s** - A predicted structural residue (highly conserved and buried).