

Supplemental information

Sequences of constructs

Nucleotide and amino acid sequences of GlpQ constructs from *B. miyamotoi* are given below. Expression vectors pET28b(+), pRSET-B, or pET24d were used. The resulting recombinant proteins carry either a C- or an N-terminal 6xHis-tag or, in the case of pET24d, an N-terminal 10xHis-tag. Sites for restriction endonucleases are underscored. The starting codon (methionine) and the His-tag are marked by bold letters.

pET28b(+)_Bm_GlpQ-C

```
1 CCATGGCCTT TAAACAAGAA ATGGGTGAAA AAAAAAGTC ACCATTGATC ATAGCTCACA
61 GGGGTGCTAG TGGATACCTT CCAGAACATA CCTTAGAAGC TAAAGCATAT GCTTATGCCT
121 TAGGAGCTGA TTATCTAGAA CAAGACATAG TTCTAACAAA GGACAATATT CCTGTTATAA
181 TGCACGACCC AGAAATTGAC ACAACCACAA ATGTTGCACA ATTATTTCCC AATCGAGCTA
241 GAGAAAACGG AAGATATTAC GCTACTGACT TCACACTAAC TGAACTTAAA TCACTAAATC
301 TCAGTGAAAG ATTTGATCCT GAAAATAAAA AACCAATATA TCCTAATCGT TTTCCCTTAA
361 ATGAATATAA TTTTAAAATT CCAACTTAG AAGAAGAAAT ACAATTCATA CAAGGACTAA
421 ATAAAAGCAC AGGAAAGAAT GTTGGGATTT ACCCTGAGAT TAAAAAACCC TTCTGGCATA
481 AACACAAGG TAAAGATATC TCTAAAATTG TAATAGAAAT TTTAAAATAA TATGGGTATA
541 AATCAAAAGA AGATAAGATT TACCTACAAA CATTGCACTT TGATGAATTA AAAAGAATAA
601 GAAAAGAACT TGGATACCAA GGCAAATTAA TAATGCTTGT TGGAGAAAAT GACTGGAATG
661 AAGCACCAAC AGACTATGAA TATATAAAAT CAGAAGAAGG TATTGCTGAA GTTGCAAAAT
721 ATTCTGATGG AATTGGACCC TGGATACCCC AAATTATAAT TGATGGTAAA ATAACAGAAC
781 TTACAACTT AGCACATAAA TATAATATAG AGGTTTATCC TTATACATTT AGGACAGATG
841 CATTACCTTC ATATGTTAAA AATGAAAATG AATTATTAGA TTTATTATTT AACAAAGCAA
901 AAGTAGATGG TATATTTACA GATTTTACTG ACACAGTAAT GAATTTTATA AAAAAAGAAT
961 TCCTTGAAGT TCTTTTTTCAA GGTCTCACC ACCACCACCA CCACGAATTC TAAAAGCTT
```

```
1 MAFKQEMGEN KKSPLIIAHR GASGYLPEHT LEAKAYAYAL GADYLEQDIV LTKDNIPVIM
61 HDPEIDTTN VAQLFPNRAR ENGRYYATDF TLTELKSLNL SERFDPENKK PIYPNRFPLN
121 EYNFKIPTLE EEIQFIQGLN KSTGKNVGIY PEIKKPFWHK QQKDISKIV IEILNKYGYK
181 SKEDKIYLTQ FDFDELKRIR KELGYQGKLI MLVGENDWNE APTDYEYIKS EEGIAEVAKY
241 SDGIGPWIPQ IIDGKITEL TNLAHKYINIE VHPYTFRTDA LPSYVKNENE LLDLLENKAK
301 VDGIFTDFTD TVMNFIKKEF LEVLVQGPHH HHHHEF
```

pRSET-B_Bm_GlpQ-N

```
1 ATGCGGGGTT CTCATCATCA TCATCATCAT GGTATGGCTA GCATGACTGG TGGACAGCAA
61 ATGGGTGCGG ATCTGTACGA CGATGACGAT AAGGATCCGA GCTCGAGATC TGCAGCTGGT
121 ACCATGGCCT TTAACAAGA AATGGGTGAA AACAAAAAGT CACCATTGAT CATAGCTCAC
181 AGGGGTGCTA GTGGATACCT TCCAGAACAT ACCTTAGAAG CTAAAGCATA TGCTTATGCC
241 TTAGGAGCTG ATTATCTAGA ACAAGACATA GTTCTAACAA AGGACAATAT TCCTGTTATA
301 ATGCACGACC CAGAAATTGA CACAACCACA AATGTTGCAC AATTATTTCC CAATCGAGCT
361 AGAGAAAACG GAAGATATTA CGCTACTGAC TTCACACTAA CTGAACTTAA ATCACTAAAT
421 CTCAGTAAA GATTTGATCC TGAAAATAAA AAACCAATAT ATCCTAATCG TTTTCCCTTA
481 AATGAATATA ATTTTAAAAT TCCAACCTTA GAAGAAGAAA TACAATTCAT ACAAGGACTA
541 AATAAAAGCA CAGGAAAGAA TGTTGGGATT TACCCTGAGA TTAATAAAACC CTCTGGCAT
601 AAACAACAAG GTAAAGATAT CTCTAAAATT GTAATAGAAA TTTTAAATAA ATATGGGTAT
661 AAATCAAAAG AAGATAAGAT TTACTACAA ACATTCGACT TTGATGAATT AAAAAGATA
721 AGAAAAGAAC TTGGATACCA AGGCAAATTA ATAATGCTTG TTGGAGAAA TGGAGGAAT
781 GAAGCACCAA CAGACTATGA ATATATAAAA TCAGAAGAAG GTATTGCTGA AGTTGCAAAA
841 TATTCTGATG GAATTGGACC CTGGATACCC CAAATTATAA TTGATGGTAA AATAACAGAA
901 CTTACAAACT TAGCACATAA ATATAATATA GAGGTTTATC CTTATACATT TAGGACAGAT
961 GCATTACCTT CATATGTTAA AAATGAAAAT GAATTATTAG ATTTATTATT TAACAAAGCA
```

1021 AAAGTAGATG GTATATTTAC AGATTTTACT GACACAGTAA TGAATTTTCAT AAAAAAAGAA
 1081 TTCTAAAAGC TT

1 **MRGSHHHHHH** GMASMTGGQQ MGRDLYDDDD KDPSSRSAAG TMAFKQEMGE NKSPLIIAH
 61 RGASGYLPEH TLEAKAYAYA LGADYLEQDI VLTKNIPVI MHDPEIDTTT NVAQLFPNRA
 121 RENGRIYATD FTLTELKSLN LSERFDPENK KPIYPNRFPL NEYNFKIPTL EEEIQFIQGL
 181 NKSTGKNVGI YPEIKKPFWH KQQGKDISKI VIEILNKYGY KSKEDKIYQL TFDDELKRI
 241 RKELGYQGKL IMLVGENDWN EAPTDYEYIK SEEGIAEVAK YSDGIGPWIP QIIIDGKITE
 301 LTNLAKHYNI EVHPYTFRTD ALPSYVKNEN ELLDLLFNKA KVDGIFTDFT DTMVNFIKKE
 361 F

pET24d_GlpQ_{DIA}

1 **CCATGGCACA TCACCATCAC CATCACCATC ACCATCACTT** TAAGCAGGAA ATGGGCAGCA
 61 ATAAAAAGAG CCCGCTGATT ATCGCGCACC GTGGCGCGAG CGGCTACCTG CCAGAACATA
 121 CGCTGGAGGC AAAGGCATAC GCCTATGCGC TGGGCGCAGA CTACCTGGAA CAGGACATCG
 181 TGCTGACCAA AGATAATATC CCGGTGATCA TGCACGATCC TGA AATTGAC ACGACCACTA
 241 ACGTCGCCCA GCTCTTCCCG AACCGTGCTC GCGAAAACGG CCGTTACTAT GCTACTGATT
 301 TCACCCTGAC TGAAC TGA AAA TCCCTCTCCC TGTCTGAACG TTTCGACCCA GAAAATAAAA
 361 AGCCGATCTA CCCAAACCGC TTCCCGCTGA ACGAGTACAA TTTCAAAATC CCGACCTGG
 421 AGGAAGAGAT TAAATTCATC CAAGGCCTCA ACAAATCGAC CGGTCGCAAC GTAGGTATCT
 481 ACCCGGAAAT TAAAAAGCCT TTTTGGCATA AACAGCAAGG CAAGGATATC AGCAAAAATTG
 541 TTATCGAAAT TCTGAACAAA TATGGTTATA AATCCAAAAGA AGATAAAAATC TACCTGCAGA
 601 CTTTCGATTT CGATGAACTC AAGCGCATTC GTAAAGAACT GGGTTACCAG GGTAAACTTA
 661 TCATGCTGGT AGGTGAAAAC GATTGGAATG AGGCTCCGAC GGACTATGAG TATATTTAAAT
 721 CCGAGGAAGG CATTGCAGAA GTGGCTAAAT ACTCTGACGG TATCGGCCCG TGGATCCCGC
 781 AGATCATTAT CGACGGCAAA ATTACTGAAC TGACCTCTCT GGCCATAAAA TATAACATTG
 841 AGGTACACCC GTACACCTTC CGTACCGACT CTCTGCCATC CTACGTTAAA AATGAGAACG
 901 AACTTCTGGA CCTGCTGTTT AACAAAGCGA AAGTCGACGG CATCTTTACT GATTTCACTG
 961 ACACCGTTAT GAACTTTTAT AAAAAATGAT AAGAATTC

1 **MAHHHHHHHH** HHFKQEMGSN KKSPLIIAHR GASGYLPEHT LEAKAYAYAL GADYLEQDIV
 61 LTKDNIPVIM HDPEIDTTTN VAQLFPNRAR ENGRYATDF TLTELKLSL SERFDPENKK
 121 PIYPNRFPLN EYNFKIPTLE EEIKFIQGLN KSTGRNVGIY PEIKKPFWHK QQKDISKIV
 181 IEILNKYGYK SKEDKIYQLT FDFDELKRIR KELGYQGKLI MLVGENDWNE APTDYEYIKS
 241 EEGIAEVAKY SDGIGPWIPQ IIDGKITEL TSLAHKYNIE VHPYTFRTDS LPSYVKNENE
 301 LLDLLFNKAK VDGIFTDFTD TVMNFIIK

Pairwise alignment of GlpQ protein sequences used in the Vienna and DIARECT laboratories

	5	15	25	35	45	55
pRSET-B_Bm_GlpQ	MRGSHHHHHH	GMASMTGGQQ	MGRDLYDDDD	KDPSSRSAAG	TMAFKQEMGE	NKSPLIIAH
pET24d_GlpQ _{DIA}	MAHHHHHHHH	-----	-----	-----	-HH.....S

	65	75	85	95	105	115
pRSET-B_Bm_GlpQ	RGASGYLPEH	TLEAKAYAYA	LGADYLEQDI	VLTKNIPVI	MHDPEIDTTT	NVAQLFPNRA
pET24d_GlpQ _{DIA}

	125	135	145	155	165	175
pRSET-B_Bm_GlpQ	RENGRIYATD	FTLTELKSLN	LSERFDPENK	KPIYPNRFPL	NEYNFKIPTL	EEEIQFIQGL
pET24d_GlpQ _{DIA}SK.....

	185	195	205	215	225	235

```

pRSET-B_Bm_GlpQ   NKSTGKNVGI YPEIKKPFWH KQQGKDISKI VIEILNKYGY KSKEDKIYLO TDFDFELKRI
pET24d_GlpQDia   .....R.....

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                245      255      265      275      285      295
pRSET-B_Bm_GlpQ   RKELGYQGKL IMLVGENDWN EAPTDYEYIK SEEGIAEVAK YSDGIGPWIP QIIIDGKITE
pET24d_GlpQDia   .....

                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                305      315      325      335      345      355
pRSET-B_Bm_GlpQ   LTNLAHKYNI EVHPYTFRTD ALPSYVKKEN ELLDLLFNKA KVDGIFTDFT DTVMNFIKKE
pET24d_GlpQDia   ..S..... S.....

.

pRSET-B_Bm_GlpQ   F
pET24d_GlpQDia   -

```

Nucleotide- and amino acid sequences of Vsp1 of *B. miyamotoi* strain LB-2001. Expression vector pRSET-B was used for adding the His-tag at the N-terminus. Sites for restriction endonucleases are underscored. The starting codon (methionine) and the His-tag are marked by bold letters.

pRSET-B-Bm_Vsp1

```

  1 ATGCGGGGTT CTCATCATCA TCATCATCAT GGTATGGCTA GCATGACTGG TGGACAGCAA
 61 ATGGGTCGGG ATCTGTACGA CGATGACGAT AAGGATCCAT CATGTGGAAG TGGGGGACCG
121 GCACCTAAGG AAGGGCAGGC AGCAAAGGCT GATGGGACAG TCGTTGATTT AGTAAAAAGTA
181 AGTAAAAAGA TAAAAGATGC GGTTGAGTTT GCAGCAAATG TAAAAGAAGT AGAGACTTTA
241 GTTAAGTCAA TAAATGAACT TGCTAAAGCT ATTGGAAGA AAATTAAGTC CGATGGGCAG
301 TTTGATACTG AGTCAGGTAA AAATGGATCA TTGCTTGCAG GAGCACAAAG TATAATGTTA
361 GCTGTAAAAG CTAAGTTAGG ACAATTGGAT AATAAAGAAG GGATTTCTAC TGAAGTAAAG
421 CAAAAGGTTA CTGATTCTAA GACAAAAACA GAACTTTCT TAACTAAAT TGAAGACAAT
481 CACTCTGATC TTGGTAAAAA TGAAGCTACT GATGCTCATG CAAAAAGTGC TATAGATATA
541 ACTGATACTG GTACTAAAGA TAAAGGAACT TCTGAGCTTA TCGCTTTGAA TACATCAATT
601 AATGCTTTGT TAGAAACCGC TAATGATGAA GTAGAAGCCG CAATTAAGGC CCTTATAAAT
661 CCTTCTAAAG CATTAACTGC TGGTCAATCT TCATAATGTA AAGAGATAAA TAATTTGAGT
721 AATTGTTATA AGTTTAAATTT TTAATTAATAA GGTAAGTAAC TGGGAAACTC GAG

```

```

  1 MRGSHHHHHH GMASMTGGQQ MGRDLYDDDD KDPSCGSGGP APKEGQAAKA DGTVVDLVKV
 61 SKKIKDAVEF AANVKEVETL VKSINELAKA IGKKIKSDGQ FDTESGKNGS LLAGAQSIML
121 AVKAKLGQLD NKEGISTELK QKVTDKTKT ETFLTKLKDN HSDLGKNEAT DAHAKSAIDI
181 TDTGTKDKGT SELIALNTSI NALLETANDE VEAAIKALIN PSKALTAGQS S

```

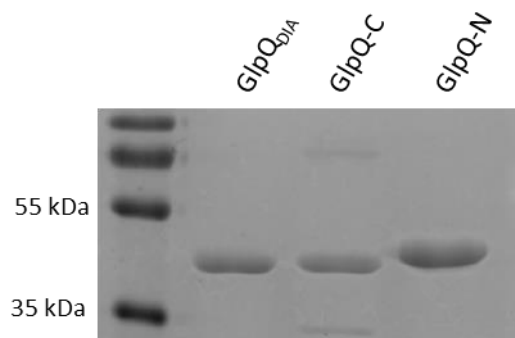


Figure S1. SDS-polyacrylamide gel electrophoresis of the different versions of GlpQ produced by us followed by staining with Coomassie Brilliant Blue. From left to right: GlpQ_{DIA} produced by DIARECT (38 kDa); GlpQ-C, GlpQ carrying a C-terminal 6xHis tag (~39 kDa); GlpQ-N, GlpQ carrying an N-terminal 6xHis tag (~41 kDa) both produced by the Vienna laboratory.

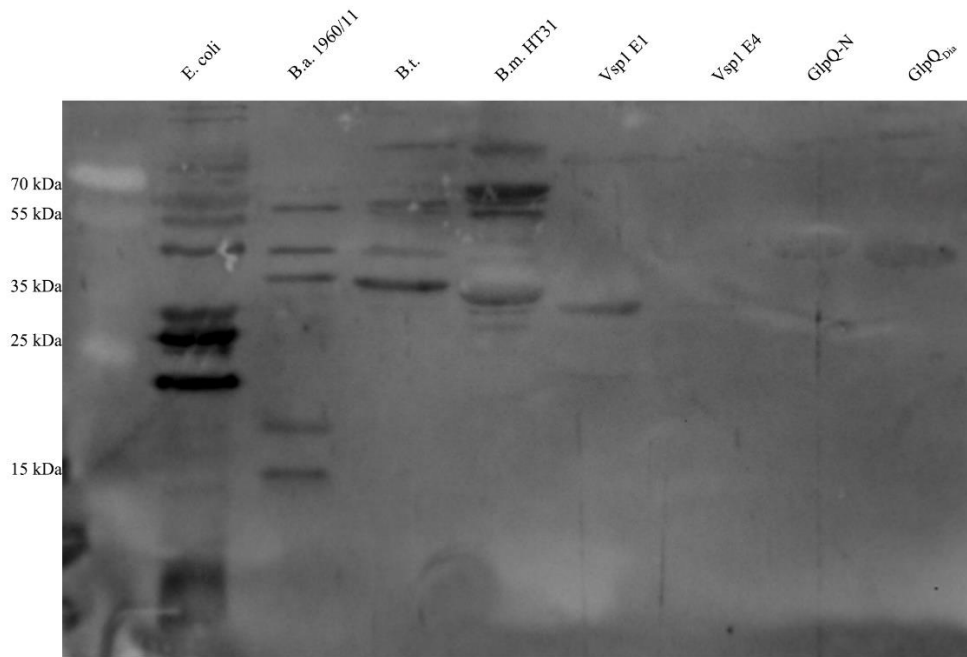


Figure S2. Immunoblot with the serum of the LNB patient, whose IgG shows weak reactivity against GlpQ. Bands visible for Vsp1 E1 and E4 are artefacts, probably due to impurities in the protein isolation (genuine bands for Vsp1 should be visible at ~24 kDa). *E. coli* strain B121(DE3)pLysS; B.a., *B. afzelii* strain 1960/11; B.t., *B. turicatae*; B.m.; HT31, *B. miyamotoi* strain HT31; Vsp1(E1 and E4), Vsp1 isolations with different purities; GlpQ-N, N-terminally 6xHis-tagged version of GlpQ produced in the Vienna laboratory; GlpQ_{DIA}, recombinant GlpQ produced by DIARECT.

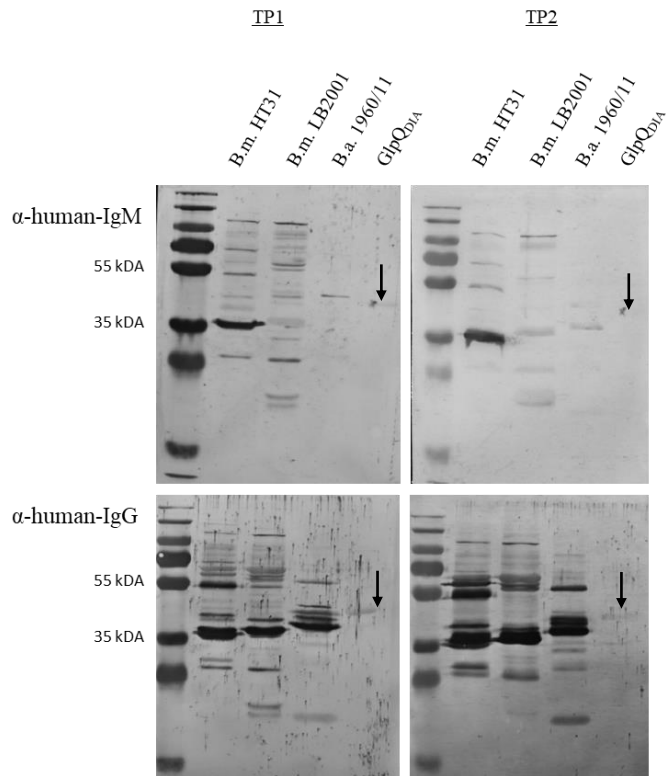


Figure S3. Serum samples of a *B. miyamotoi*-infected person from two different time points (TP). Infection was PCR confirmed at TP2. Sample collection at TP1 (13 weeks earlier) was not related to *B. miyamotoi* infection. The upper row was probed with α -human-IgM antibodies. The lower row was probed with α -human-IgG antibodies. B. m. HT31, *B. miyamotoi* strain HT31; B. m. LB2001, *B. miyamotoi* strain LB-2001; B.a. 1960/11, *B. afzelii* strain 1960/11; GlpQ_{DIA}, recombinant GlpQ produced by DIARECT. Arrows indicate the correct height of GlpQ_{DIA}