

Supplementary Material

Supplementary Figure 1: Mass spectrometry analysis of purified BadA from *B. henselae* Marseille. For immunisation of rabbits, fibers from *B. henselae* Marseille were purified (see Material and Methods) and the HMW protein was identified as BadA using mass spectrometry. Grey highlighted stretches in the BadA protein sequence are mass spectrometry-identified peptides.

MKKLSVTSKRQYNLYASPI SRRLSLLMKLSLETVTVMFLLGASPVLASNLALTGAKNLSQNSPGVNYSKGSHGSI VLVSGD
DDFCGADYVLRGGNSTVRNGIPISVEEYERFVKQKLMNNATSPYSQSSEQQVWTGDGLTSKSGSYMGGKSTDGDKNLL
PEAYGYSFATGCGSSAQGNYSVAFGANATALTGGSQAFGVAALASGRVSVAI GVGSEATGEAGVSLGGLSKAAGARSVAI
IGTRAKAQGEESIAIGSSVKNKGDKGS AVAQGAKAIATIGSNSISFQHYAVAVGAKAHALLSKTVALGYDSVADV DAGIRG
YDPVEDEPSKDVSEFVWKSLLGAVSVGNRKEGLTRQIIGVAAGTEDTDAVNVAQLKALRGMISEKGGWNLTVNNNDNNTVVS
SGGALDLSGSKNLIKIVKDGKKNVTFDVARDLTLKSIKLDGVTLNETGLFIANGPQITASGINAGSQITGVAEGTDAN
DAVNFGQLKKIETEVEKQVAASGFVKQSDSTKYLTIGKDTDGTINIANNKSKDRTLTGKIGEDI SKDSSEAITGSQFLT
TNQNVKTVSDNLQTAATNIAKTFGGGAKYEDGEWIAPAFKVKTVTGEKKEEKRYQNADALAGVSSITNVQNKVTEQV
NNAITKVEGDALLWSDEANAFVARHEKSKLGGKASKATQENSKI TYLLDGDVSKDSTDAITGKQLYSLGDKIASYLGNA
KYEDGEWTAPTFFKVKTVKEDGKEEKTYQNVAEALTVGTSFTNVKNEITKQINHLQSDDSAVVHYDKNKDETTGGINYS
VTLGKGDSDAAVTLHNVADGSI SKDSRDAINGSQIYSLNEQLATYFGGGAKYENGQWTAPIFKVKTVKEDGEEEEKTYQN
VAEALTVGTSFTNFKSEITKQIANEISSVTGDSL VKKDLATNLITIGKEVAGTEINIASVSKADRTLSGVKEAVKDNEA
VNGQLDKGLKHLSDSLQSDDSAVVHYDKKTDDETTGGINYSVTLGGKDKTPVALHNVADGSI SKDSHDAINGGQIHTIGE
DVAKFLGGAA SFNNGAFTGPTYKLSNIDAKGDVQQSEFKDIGSAFAGLDNTNKNVNNVTNKFNELTQNI TNVTQQVKG
ALLWSDEANAFVARHEKSKLGGKASKATQENSKI TYLLDGDVSKDSTDAITGKQLYSLGDKIASYLGNAKYENGEWTA
PTFKVKTVKEDGKEEKTYQNVAEALTVGTSFTNVKNEITKQINHLQSDDSAVVHYDKNKDETTGGINYSVTLGKGD
SAVTLHNVADGSI SKDSRDAINGSQIYSLNEQLATYFGGGAKYENGQWTAPIFKVKTVKEDGEEEEKTYQNVAEALTVG
TFTNFKSEITKQIANEISSVTGDSL VKKDLATNLITIGKEVAGTEINIASVSKADRTLSGVKEAVKDNEAVNKGQLDKGL
KHLSDSLQSDDSAVVHYDKKTDDETTGGINYSVTLGGKDKTPVALHNVADGSI SKDSHDAINGGQIHTIGEDVAKFLGGAA
SFNNGAFTGPTYKLSNIDAKGDVQQSEFKDIGSAFAGLDNTNKNVNNVTNKFNELTQNI TNVTQQVKG DALLWSDEANA
FVARHEKSKLGGKASKATQENSKI TYLLDGDVSKDSTDAITGKQLYSLGDKIASYLGNAKYENGEWTAFTFKVKTVKED
GKEEEKTYQNVAEALTVGTSFTNVKNEITKQINHLQSDDSAVVHYDKNKDETTGGINYSVTLGKGDSDAAVTLHNVAD
GSI SKDSRDAINGSQIYSLNEQLATYFGGGAKYENGQWTAPIFKVKTVKEDGEEEEKTYQNVAEALTVGTSFTNFKSEIT
KQIANEISSVTGDSL VKKDLATNLITIGKEVAGTEINIASVSKADRTLSGVKEAVKDNEAVNKGQLDNTNKKVEDKLT
EAVGKVTQVKG DALLWSNEDNAFVADHGKDSAKTKSKI THLLDGNIASGSTDAVTGGQLYSLNEQLATYFGGGAKYENGQ
WTAPTFFKVKTVNGEGKEEETQYQNVAEALTVGTSFTNVKNEITKQINHLQSDDSAVVHYDKNKDETTGGINYSVTLGKGD
SAVTLHNVADGSI SKDSRDAINGSQIYSLNEQLATYFGGGAKYENGQWTAPIFKVKTVKEDGEEEEKTYQNVAEALTVG
TFTNFKSEITKQIANEISSVTGDSL VKKDLATNLITIGKEVAGTEINIASVSKADRTLSGVKEAVKDNEAVNKGQLDANI
SKVNNNVTNKFNELTQNI TNVTQQVKG DALLWSDEANAFVARHE
KSKLEKGVSKATQENSKI TYLLDGDISKGSTDAVTGGQLYSLNEQLATYFGGGAKYENGQWTAPTFFKVKTVNGEGKEE
EQTYQNVAAAFEGVGTSTFTNFKSEITKQINNEI INVKGD SLVKRDLATNLITIGKEIEG SVINIANKSGEARTISGVKEAV
KDNEAVNKGQLDNTNKKVEDKLT EAVGKVTQVKG DALLWSNEDNAFVADHGKDSAKTKSKI THLLDGNIASGSTDAVTGG
QLYSLNEQLATYFGGGAKYENGQWTAPTFFKVKTVNGEGKEEETQYQNVAAAFEGVGTSTFTNFKSEITKQIANEISNVTG
D SLVKKDLDTNLITIGKEIAGTEINIASVSKADRTLSGVKEAVNDNEAVNKGQLDANI SKVNNNVTNKFNELTQNI TNVT
QVKG DALLWSDEANAFVARHEKSKLEKGVSKATQENSKI TYLLDGDISKGSTDAVTGGQLYSLNEQLATYFGGGAKYENG
QWTAPTFFKVKTVNGEGKEEETQYQNVAAAFEGVGTSTFTNFKSEITKQINNEI INVKGD SLVKRDLATNLITIGKEIEG SV
INIANKSGEARTISGVKEAVKDNEAVNKGQLDNTNKKVEDKLT EAVGKVTQVKG DALLWSNEDNAFVADHGKDSAKTKS
KI THLLDGNIASGSTDAVTGGQLYSLNEQLATYFGGGAKYENGQWTAPTFFKVKTVNGEGKEEETQYQNVAAAFEGVGTST
FTNFKSEITKQIANEISNVTGDSL VKKDLDTNLITIGKEIAGTEINIASVSKADRTLSGVKEAVNDNEAVNKGQLDANI SK
VNNNVTNKFNELTQNI TNVTQQVKG DALLWSDEANAFVARHEKSKLEKGVSKATQENSKI TYLLDGDISKGSTDAVTGGQ
LYSLNEQLATYFGGGAKYENGQWTAPTFFKVKTVNGEGKEEETQYQNVAAAFEGVGTSTFTNFKSEITKQINNEI INVKGD
SLVKRDLATNLITIGKEIEG SVINIANKSGEARTISGVKEAVKDNEAVNKGQLDNTNKKVEDKLT EAVGKVTQVKG DALL
WSNEDNAFVADHGKDSAKTKSKI THLLDGNIASGSTDAVTGGQLYSLNEQLATYFGGGAKYENGQWTAPTFFKVKTVNGEG
KEEETQYQNVAAAFEGVGTSTFTNFKSEITKQINHLQSDDSAVVHYDKDDKNGSINYSVTLGKGDSDAAVALHNVADGSI
SKDSHDAINGGQIHTIGEDVAKFLGGDAAFKDGAFPTGPTYKLSNIDAKGDVQQSEFKDIGSAFAGLDNTNKNVNNVTN
KSELTONITVTQQVKNALLWSDEANAFVARHEKSKLEKGVSKATQENSKI TYLLDGDVSKDSTDAVTGGQLYSMSNML
ATYLGNAKYENGEWTAFTFKVKTVNGEGKEEETQYQNVAEALTVGTSFTNFKSEIAKQINHLQSDDSAVIHYDKNKDE
TGTINYSVTLGKGDSDAAVALHNVAAAGNIAKDSRDAINGSQIYSLNEQLATYFGGGAKYENGQWTAPTFFKVKTVNGEG
SGEKESYDNVAAAFEGVKNKSLAGMNERINNVTAGQNVSSSSLNWNETEGGYDARHNGVDSKLTHTVNGDVSEKSEAVNG
SQLWNTNEKVEAVEKDVKNIEKQVQDIATVADSAVKYKDSGTGKKTNVIKLVGSESEPVLDINVADGKI EADSKQAVNG
QQLRDYTEKQMKIVLDDAKKYTDERFNDVVNNGINEAKAYTDVKEALSYTVEEVRKEARQAAAIGLAVSNLRYDIPGS
LSLSFGTGIWRSQSAFAIGAGYTSSEGNIRSNLSITSSGGQWCVGAGITLRLK

Supplementary Table 1: Genome size, sequencing data, and GenBank accession number for eight sequenced *B. henselae* genomes (GC-content is 38% for all strains).

<i>B. henselae</i> strain	Single contig genome size (bp)	Mean coverage ^a	N50 subread length (bp) ^b	Subreads	Mean subread length (bp)	CCS reads (\geq Q20) ^c	CCS mean read length (\geq Q20 ^b , bp)	Predicted CDSs	GenBank accession number
Marseille	1,906,759	802x	9,778	335,562	7,486	18,542	7,486	1,566	CP072904
ATCC49882 ^T var-1	1,955,459	1,113x	9,778	631,218	5,801	28,343	5,801	1,574	CP072903
ATCC49882 ^T var-2	1,931,585	651x	10,446	306,776	8,307	10,695	6,051	1,579	CP072902
Berlin-I	1,931,655	652x	10,446	238,737	9,695	9,490	7,440	1,584	CP072901
G-5436	1,963,796	629x	10,446	255,359	9,561	9,597	7,025	1,577	CP072900
88-64 Oklahoma	1,969,298	566x	10,446	215,349	9,663	8,300	7,157	1,610	CP072899
FR96/BK38	1,944,393	762x	10,446	371,120	8,161	12,787	8,792	1,561	CP072898
FR96/BK3	1,935,288	922x	10,446	356,346	9,460	13,834	7,089	1,571	CP072897

^a Total #bases/genome size

^b for entire SMRT cell

^c no Q-filtered reads for Marseille and ATCC49882^T var-1

Supplementary Table 2: Primers used in this study ^a.

Primer	5'-3' sequence
FrUp_Fw	<u>AGCCCGGGGGATCC</u> ATTGTTTGGCAGTGTCCAAC
FrUp_Rv	CCTGCGCCTCTCTTTGATGTGACAGATAATTTTTTCATAATAAATTCTCCAA
FrDown_Fw	<u>AAAGAGAGGCGCAGGG</u> ATTACTTTGAG
FrDown_Rv	<u>GGCCGCTCTAGAACTAG</u> ACTTCAAATAATATCTCTTACTATTGAATAATATTTTCCTAAAAC
pBIISK_Fw	CTAGTTCTAGAGCGGCCGC
pBIISK_Rv	TGGATCCCCCGGGCTG
IntegrationA_Fw	GGATCCATTGGTTTGGCAGTG
IntegrationA_Rv	GTTTTCCATGGTAGCATCACTC
IntegrationB_Fw	TTTAAGCTACGCGTTGAGGATG
IntegrationB_Rv	CGCTCTAGAACTAGACTTCAAATAATATCTCTTAC
Repeat_Fw	GAGATTGCTAATGCGAATGG
Repeat_Rv	ATTACCAACACCATTGCC

^a Underlined regions create overhangs used for Gibson cloning

Supplementary Table 3: Location of the ORFs in the *badA* island (see also Fig. 2).

	Marseille	ATCC49882 ^T var-1	ATCC49882 ^T var-2	Berlin-I	G-5436	88-64 Oklahoma	FR96/BK38	FR96/BK3
<i>badA</i> pseudogene								
NCBI locus tag	KAE73_05700	KAE76_05665	KAE74_05750	KAE72_05775	KAE71_04240	KAE77_04380	KAE75_04135	KAE70_05705
Start position	1,353,514	1,383,089	1,383,227	1,383,299	945,048	949,628	923,247	1,379,217
Size (bp)	5,361	5,181	5,181	5,181	5,181	5,181	5,430	5,430
<i>badA</i>								
NCBI locus tag	KAE73_05715 and MK993576	<i>babA</i> and KAE76_05685	KAE74_05770... KAE74_05780	KAE72_05790... KAE72_05800	KAE71_04225	KAE77_04365	KAE75_04120	KAE70_05720
Start position	1,362,098	1,391,491 and 1,396,823 ^a	1,391,629	1,391,701	941,826	935,475	909,103	1,387,870
Size (bp)	11,922	5,331 and 5,331	8,763	8,762	10,926	10,932	10,926	13,224
<i>badA</i>-like domain region								
NCBI locus tag	/	KAE76_05690... KAE76_05770	/	/	KAE71_04220...KA E71_04150	/	KAE75_04115...KA E75_04045	/
Start position	/	1,402,734 ^a	/	/	930,320 ^a	/	908,530 ^a	/
Size (bp)	/	21,532	/	/	21,532	/	20,510	/

^a NCBI annotations and ORFs in Figure 2 differ slightly from each other due to manual adjustment of some ORFs.