Document S3. Relative conservation of *Bartonella* TrwG, VirB8 and VbhB8 proteins.

Intra-genome sequence conservation of VirB8 family proteins from five *Bartonella* species

Fourteen VirB8 family proteins were aligned across five *Bartonella* species: *B. grahamii* str. as4aup (VirB8, TrwG, VbhB8-1, VbhB8-2), *B. tribocorum* str. CIP 105476 (VirB8, TrwG, VbhB8), *B. birtlesii* str. LL-WM9 (VirB8, TrwG, VbhB8), *B. henselae* str. Houston-1 (VirB8, TrwG), and *B. quintana* str. Toulouse (VirB8, TrwG). Complete proteins were aligned using MUSCLE (default parameters) (Edgar 2004), with TMHMM v.2.0 (Krogh et al. 2001) used to demarcate the boundaries between the cytoplasmic/TMS region (N-terminal domain) and periplasmic C-terminal domain of all proteins. Percent identity matrices (calculated by MUSCLE) were used to provide estimates of divergence across full-length sequences, as well as the NT and CT domains (**Table 1**). NCBI GenBank accession numbers for all proteins are provided in **Document S1**.

Conservation of the N-terminal/TMS region of *Bartonella* TrwG, VirB8 and VbhB8 proteins

Multiple sequence alignment of the NT sequences and complete TMS regions for fourteen VirB8 family proteins across five *Bartonella* species (**fig 1**). Sequences are those analyzed in **Table 1** above. Predicted TMS regions are colored blue. Highlighting as follows: yellow, conserved across TrwG, VirB8 and VbhB8 proteins; orange, conserved in TrwG and VirB8 proteins; green, conserved in VirB8 and VbhB8 proteins.

REFERENCES

Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

Krogh A, Larsson B, von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J. Mol. Biol. [Internet] 305:567–580. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11152613

Table 1 (Document S3)

Table 1 (Document S3). Intra-genome sequence conservation of VirB8 family proteins from five *Bartonella* species.

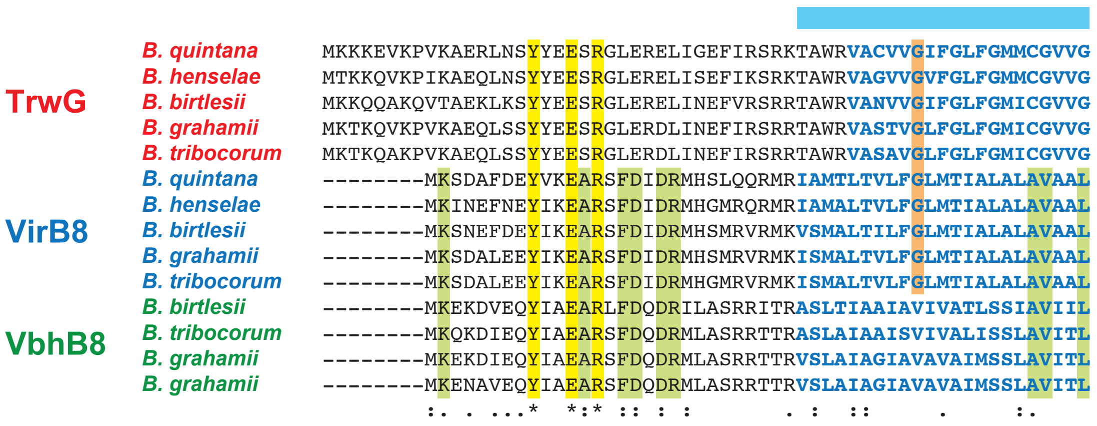
	VirB8 *vs.* TrwG [1]			**TrwG *vs.* VbhB8** [1]			**VirB8 *vs.* VbhB8** [1]			**VbhB8-1 *vs.* VbhB8-2** [1]		
	All [2]	**NTD** [3]	**CTD** [4]	**All** [2]	**NTD** [3]	**CTD** [4]	**All** [2]	**NTD** [3]	**CTD** [4]	**All** [2]	**NTD** [3]	**CTD** [4]
B. grahamii	29.4	15.4	35.3	28.8	15.4	34.5	57.7	42.3	62.4	88.8	94.2	87.1
B. tribocorum	29.4	15.4	35.3	28.8	17.3	33.9	59.0	40.4	64.1	------	------	------
B. birtlesii	25.3	7.7	32.3	30.2	17.3	35.7	57.2	30.8	64.7	------	------	------
B. henselae	28.1	13.5	34.1	------	------	------	------	------	------	------	------	------
B. quintana	27.2	11.5	33.5	------	------	------	------	------	------	------	------	------

[1] % amino acid identity, as calculated from a percent identity matrix across alignments.

[2] Entire protein.

[3] NT-domain, which includes cytoplasmic region and predicted transmembrane-spanning region.

[4] CT-domain, which includes short unstructured periplasmic region and globular domain.



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       B. quintana     MKKKEVKPVKAERLNSYYEESRGLERELIGEFIRSRKTAWRVACVVGIFGLFGMMCGVVG
       B. henselae     MTKKQVKPIKAEQLNSYYEESRGLERELISEFIKSRKTAWRVAGVVGVFGLFGMMCGVVG
TrwG   B. birtlesii    MKKQQAKQVTAEKLKSYYEESRGLERELINEFVRSRRTAWRVANVVGIFGLFGMICGVVG
       B. grahamii     MKTKQVKPVKAEQLSSYYEESRGLERDLINEFIRSRRTAWRVASTVGLFGLFGMICGVVG
       B. tribocorum   MKTKQAKPVKAEQLSSYYEESRGLERDLINEFIRSRRTAWRVASAVGLFGLFGMICGVVG
       B. quintana     --------MKSDAFDEYVKEARSFDIDRMHSLQQRMRIAMTLTVLFGLMTIALALAVAAL
       B. henselae     --------MKINEFNEYIKEARSFDIDRMHGMRQRMRIAMALTVLFGLMTIALALAVAAL
VirB8  B. birtlesii    --------MKSNEFDEYIKEARSFDIDRMHSMRVRMKVSMALTILFGLMTIALALAVAAL
       B. grahamii     --------MKSDALEEYIKEARSFDIDRMHSMRVRMKISMALTVLFGLMTIALALAVAAL
       B. tribocorum   --------MKSDALEEYIKEARSFDIDRMHGMRVRMKISMALTVLFGLMTIALALAVAAL
       B. birtlesii    --------MKEKDVEQYIAEARLFDQDRILASRRITRASLTIAAIAVIVATLSSIAVIIL
VbhB8  B. tribocorum   --------MKQKDIEQYIAEARSFDQDRMLASRRTTRASLAIAAISVIVALISSLAVITL
       B. grahamii     --------MKEKDIEQYIAEARSFDQDRMLASRRTTRVSLAIAGIAVAVAIMSSLAVITL
       B. grahamii     --------MKENAVEQYIAEARSFDQDRMLASRRTTRVSLAIAGIAVAVAIMSSLAVITL
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Fig. 1 (Document S3)