Supplementary document S4. Structural modeling of VirB8 family proteins.

Structural alignment for eight proteins of the VirB8 family

For structural modeling, target sequences were computationally aligned to a set of templates and manually curated. The template alignment was constructed with eight structures downloaded from the Protein DataBank (Berman et al. 2000): 4MEI, VirB8 of *Bartonella tribocorum*; 2BHM, VirB8 of *Brucella suis* (Terradot et al. 2005); 4LSO, VirB8 of *Bartonella quintana*; 4JF8, TrwG of *Bartonella birtlesii*; 4KZ1, VirB8 of *Bartonella grahamii*; 2CC3, VirB8 of *Agrobacterium tumefaciens* (Bailey et al. 2006); 4NHF (chain A), TrwG of *Bartonella grahamii*; 4O3V, RvhB8-II of *Rickettsia typhi* (**fig. 1**). (*A*) Structural alignment generated using SPDBV/Deep View (Guex and Peitsch 1997). Sequence coordinates are described in panel B. Magenta cylinders and gray arrows depict the α-helices and β-strands for the VirB8 structure of *Bartonella tribocorum*, with highlighted regions depicting the conserved regions utilized to align target sequences to the templates (see Materials and Methods for further information). (*B*) Protein sequences retrieved from UniProt that correspond to the structures shown in panel *A*. Red coloring depicts the portion of the protein included in each respective structure, with regions shown in the alignment in bold. Yellow highlighting depicts regions from the *B*. *suis* and *R*. *typhi* proteins that are absent from the structures.

Analysis of a putative RvhB8-I/RvhB8-II heterodimer

Examination of a potential heterodimer formed by *R*. *typhi* RvhB8-I (YP_067242, modeled structure) and RvhB8-II (YP_067240, PDBID: 4O3V) proteins (**fig. 2**). See text for explanation of protein modeling procedures. (*A*) Ribbon representation of modeled RvhB8-I/RvhB8-II heterodimer. The major dimerization site involving residues of α-helix α1 and the 'NPxG' motif is boxed and enlarged at right. RvhB8-II residues are denoted with primes. Note: the minor dimerization site involving residues within the loop between α-helices α1 and α2 is not supported in this model. (*B*) Sequence alignment of *R*. *typhi* RvhB8-I and RvhB8-II proteins, with secondary structure assignment. Sequences are the globular domains depicted in panel *A*. For each protein, residues involved in the dimerization interface are within black boxes. Invariant residues are highlighted yellow. Magenta cylinders and gray arrows depict the α-helices and β-strands for the RvhB8-I and RvhB8-II structures (see **fig. 5** of the manuscript). For RvhB8-II, the brown star denotes the break in the structure, with missing residues colored white. Note: residues involved in the minor dimerization site of the RvhB8-I and RvhB8-II homodimers are highlighted green.

Assessing the structures of VirB8 family proteins lacking a conserved 'NPxG' motif

Structural models generated for 16 VirB8 sequences exhibiting divergent 'NPxG' motifs within the major dimerization site (**fig. 3**). See text for explanation of protein modeling procedures. (*A*) N**W**DG of LvhB8, *Legionella longbeachae* D-4968 (EEZ95948); (*B*) N**L**LG of hypothetical protein XPN_1731, *Xanthomonas arboricola* pv. pruni MAFF 301427 (GAE59825); (*C*) N**G**MG of TriG, *Escherichia coli* TW00353 (EKI31743); (*D*) N**D**IG of VirB8, *Pseudomonas putida* LD209 (AHC05756); (*E*) N**I**FG of hypothetical protein F892_00045, *Acinetobacter* sp. NIPH 2168 (ENX24895); (*F*) NPT**D** of competence protein, *Helicobacter pylori* Hp A-17 (EJB46117); (*G*) N**S**LG of VirB8, *Ralstonia solanacearum* CMR15 (CBJ36145); (*H*) N**S**RG of VirB8, *Pseudomonas syringae* pv. syringae SM (EPF64149); (*I*) **R**PTG of VirB8, *Hydrogenophaga* sp. T4 (EWS64160); (*J*) **T**PI**S** of hypothetical protein CPS_0790, *Colwellia psychrerythraea* 34H (AAZ25142); (*K*) NPH**L** of conjugal transfer protein TrbF, *Legionella pneumophila* Corby (YP_001249519); (*L*) N**G**TG of type IV secretion protein, *Burkholderia multivorans* CGD1 (WP_006402582); (*M*) N**A**LG of VirB8, *Xanthomonas citri* subsp. citri (NP_942615); (*N*) NPL**A** of conjugative transfer protein, *Stenotrophomonas maltophilia* EPM1 (WP_005410125); (*P*) N**Y**FG of VirB8, *Yersinia pestis* biovar Microtus str. 91001 (NP_995427); note, this structure is shown in **fig. 6*G*** of the manuscript. Residues in bold define all of these 'NPxG' variants.

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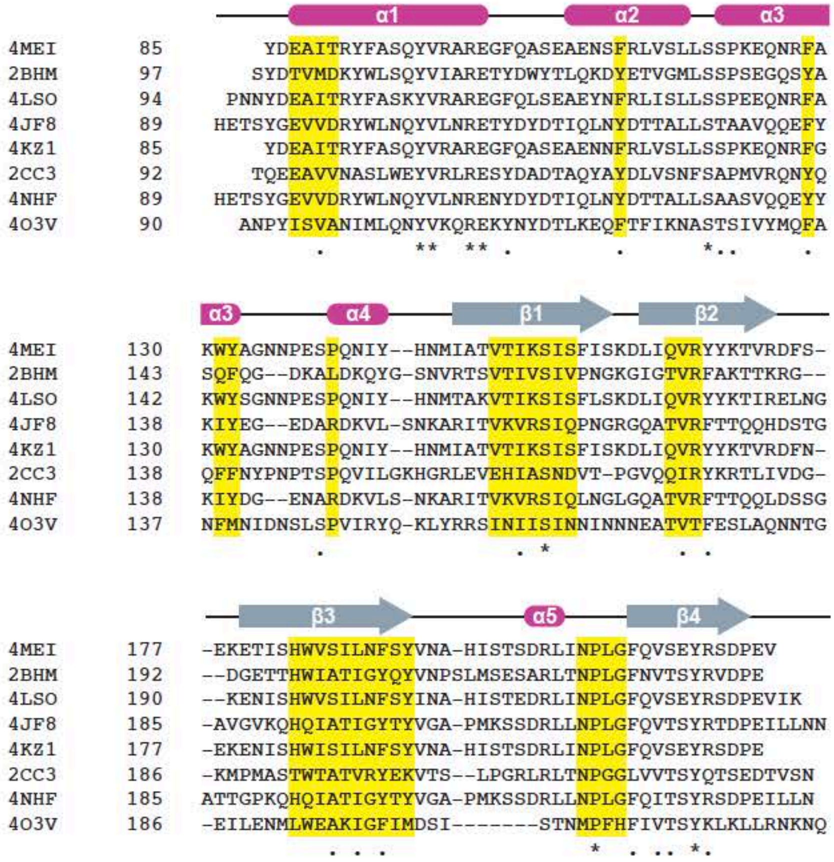
Fig. 1 (Document S4)

A



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2BHM   97  SYDTVMDKYWLSQYVIARETYDWYTLQKDYETVGMLSSPSEGQSYA
4LSO   94 PNNYDEAITRYFASKYVRAREGFQLSEAEYNFRLISLLSSPEEQNRFA
4JF8   89 HETSYGEVVDRYWLNQYVLNRETYDYDTIQLNYDTTALLSTAAVQQEFY
4KZ1   85   YDEAITRYFASQYVRAREGFQASEAENNFRLVSLLSSPKEQNRFG
2CC3   92  TQEEAVVNASLWEYVRLRESYDADTAQYAYDLVSNFSAPMVRQNYQ
4NHF   89 HETSYGEVVDRYWLNQYVLNRENYDYDTIQLNYDTTALLSAASVQQEYY
4O3V   90   ANPYISVANIMLQNYVKQREKYNYDTLKEQFTFIKNASTSIVYMQFA
                .       **  ** .        .       *..     .

4MEI  130 KWYAGNNPESPQNIY--HNMIATVTIKSISFISKDLIQVRYYKTVRDFS-
2BHM  143 SQFQG--DKALDKQYG-SNVRTSVTIVSIVPNGKGIGTVRFAKTTKRG--
4LSO  142 KWYSGNNPESPQNIY--HNMTAKVTIKSISFLSKDLIQVRYYKTIRELNG
4JF8  138 KIYEG--EDARDKVL-SNKARITVKVRSIQPNGRGQATVRFTTQQHDSTG
4KZ1  130 KWYAGNNPESPQNIY--HNMIATVTIKSISFISKDLIQVRYYKTVRDFN-
2CC3  138 QFFNYPNPTSPQVILGKHGRLEVEHIASNDVT-PGVQQIRYKRTLIVDG-
4NHF  138 KIYDG--ENARDKVLS-NKARITVKVRSIQLNGLGQATVRFTTQQLDSSG
4O3V  137 NFMNIDNSLSPVIRYQ-KLYRRSINIISINNINNNEATVTFESLAQNNTG
                .              . *         . .

4MEI  177 -EKETISHWVSILNFSYVNA-HISTSDRLINPLGFQVSEYRSDPEV
2BHM  192 --DGETTHWIATIGYQYVNPSLMSESARLTNPLGFNVTSYRVDPE
4LSO  190 --KENISHWVSILNFSYINA-HISTEDRLINPLGFQVSEYRSDPEVIK
4JF8  185 -AVGVKQHQIATIGYTYVGA-PMKSSDRLLNPLGFQVTSYRTDPEILLNN
4KZ1  177 -EKENISHWISILNFSYVNA-HISTSDRLINPLGFQVSEYRSDPE
2CC3  186 -KMPMASTWTATVRYEKVTS--LPGRLRLTNPGGLVVTSYQTSEDTVSN
4NHF  185 ATTGPKQHQIATIGYTYVGA-PMKSSDRLLNPLGFQITSYRSDPEILLN
4O3V  186 -EILENMLWEAKIGFIMDSI-------STNMPFHFIVTSYKLKLLRNKNQ
                  . . .                 *  . .. *.
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B

>tr|A9IWN6|A9IWN6_BART1 VirB8 protein OS=Bartonella tribocorum (strain CIP 105476 / IBS 506)
MKSDALEEYIKEARSFDIDRMHGMRVRMKISMALTVLFGLMTIALALAVAALTPLKTVEPFVIRVDNSTGIIDTVSALKESPSD**YDEAITRYFASQYVRARE**
GFQASEAENSFRLVSLLSSPKEQNRFAKWYAGNNPESPQNIYHNMIATVTIKSISFISKDLIQVRYYKTVRDFSEKETISHWVSILNFSYVNAHISTSDRLI
NPLGFQVSEYRSDPEVIK

>sp|Q7CEG3|VIRB8_BRUSU Type IV secretion system protein virB8 OS=Brucella suis biovar 1 (strain 1330)
MFGRKQSPQKSVKNGQGNAPSVYDEALNWEAAHVRLVEKSERRAWKIAGAFGTITVLLGIGIAGMLPLKQHVPYLVRVNAQTGAPDILTSLDEKSV**SYDTVM**
DKYWLSQYVIARETYDWYTLQKDYETVGMLSSPSEGQSYASQFQGDKALDKQYGSNVRTSVTIVSIVPNGKGIGTVRFAKTTKRTNETGDGETTHWIATIGY
QYVNPSLMSESARLTNPLGFNVTSYRVDPEMGVVQ

>sp|Q6FYW3|VIRB8_BARQU Type IV secretion system protein virB8 OS=Bartonella quintana (strain Toulouse)
MKNSLIKIRKSLVKSDAFDEYVKEARSFDIDRMHSLQQRMRIAMTLTVLFGLMTIALALAVAALTPLKTVEPFVIRVDNSTGIIETVSALKET**PNNYDEAIT**
RYFAGKYVRAREGFQLSEAEYNFRLISLLSSPEEQNRFAKWYSGNNPESPQNIYHNMTAKVTIKSISFLSKDLIQVRYYKTIRELNGKENISHWVSILNFSY
INAHISTEDRLINPLGFQVSEYRSDPEVIK

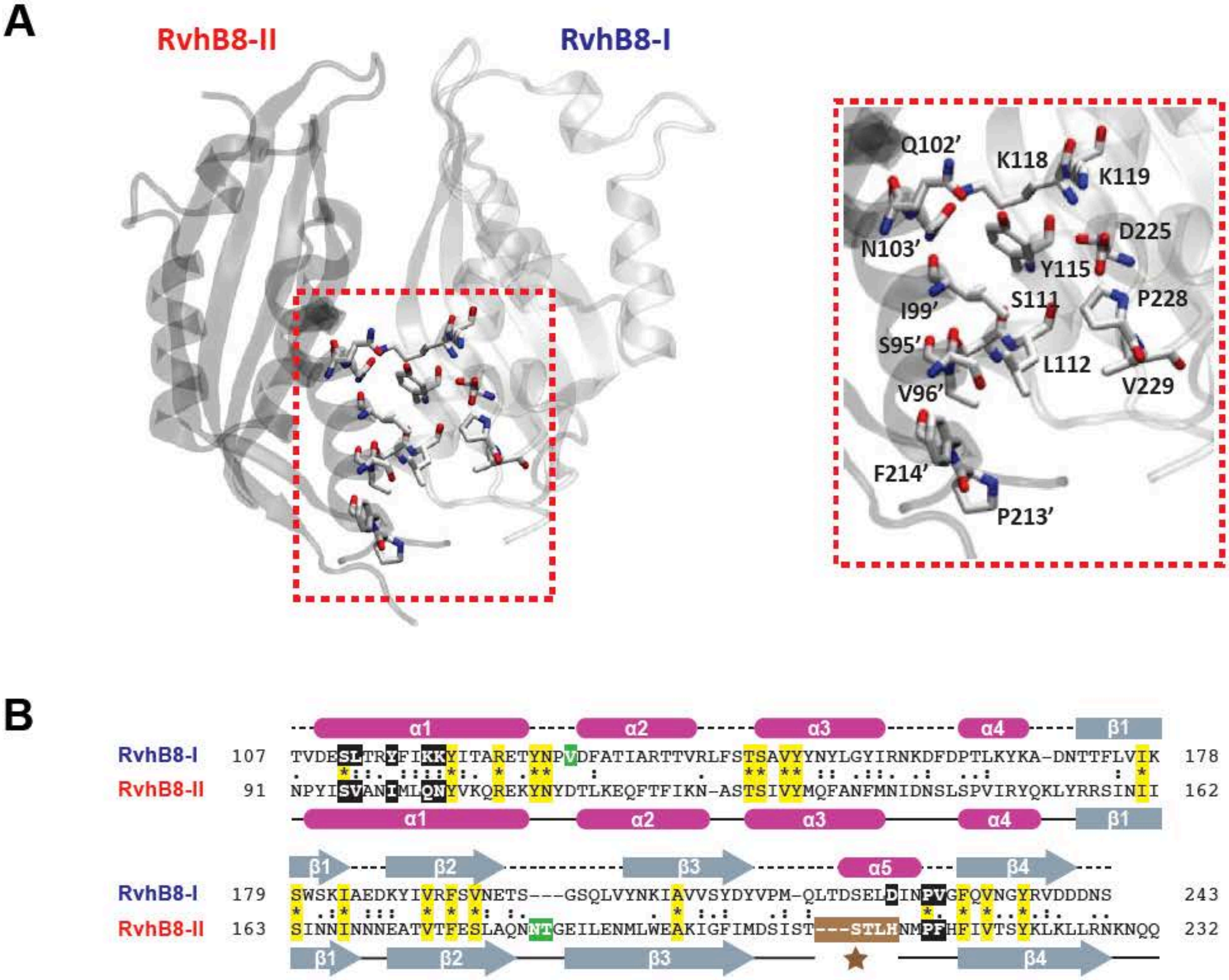
>tr|D0AAZ5|D0AAZ5_9RHIZ TrwG component of type IV secretion system OS=Bartonella birtlesii
MKKQQAKQVTAEKLKSYYEESRGLERELINEFVRSRRTAWRVANVVGIFGLFGMICGVVGFSQPAPTPLVLRVDNTTGAVDVISVMRE**HETSYGEVVDRYWL**
NQYVLNRETYDYDTIQLNYDTTALLSTAAVQQEFYKIYEGEDARDKVLSNKARITVKVRSIQPNGRGQATVRFTTQQHDSTGAVGVKQHQIATIGYTYVGAP
MKSSDRLLNPLGFQVTSYRTDPEILLNN

>tr|C6AER9|C6AER9_BARGA VirB8 protein OS=Bartonella grahamii (strain as4aup)
MKSDALEEYIKEARSFDIDRMHSMRVRMKISMALTVLFGLMTIALALAVAALTPLKTVEPFVIRVDNSTGIIDTVSALKESPND**YDEAITRYFASQYVRARE**
GFQASEAENNFRLVSLLSSPKEQNRFGKWYAGNNPESPQNIYHNMIATVTIKSISFISKDLIQVRYYKTVRDFNEKENISHWISILNFSYVNAHISTSDRLI
NPLGFQVSEYRSDPEVIK

>sp|P17798|VIRB8_AGRFC Protein virB8 OS=Agrobacterium fabrum (strain C58 / ATCC 33970)
MKGSEYALLVARETLAEHYKEVEAFQTARAKSARRLSKVIAAVATIAVLGNVAQAFTIATMVPLIRLVPVYLWIRPDGTVDSEVSVSRLPA**TQEEAVVNASL**
WEYVRLRESYDADTAQYAYDLVSNFSAPMVRQNYQQFFNYPNPTSPQVILGKHGRLEVEHIASNDVTPGVQQIRYKRTLIVDGKMPMASTWTATVRYEKVTS
LPGRLRLTNPGGLVVTSYQTSEDTVSNAGHSEP

>tr|C6AAT5|C6AAT5_BARGA TrwG protein OS=Bartonella grahamii (strain as4aup)
MKTKQVKPVKAEQLSSYYEESRGLERDLINEFIRSRRTAWRVASTVGLFGLFGMICGVVGFSQPAPTPLVLRVDNTTGAVDVISVMRE**HETSYGEVVDRYWL**
NQYVLNRENYDYDTIQLNYDTTALLSAASVQQEYYKIYDGENARDKVLSNKARITVKVRSIQLNGLGQATVRFTTQQLDSSGATTGPKQHQIATIGYTYVGA
PMKSSDRLLNPLGFQITSYRSDPEILLND

>tr|Q68X84|Q68X84_RICTY VirB8-like protein OS=Rickettsia typhi (strain ATCC VR-144 / Wilmington)
MDQLTNAIKEYIKSGEYFIDARKWYNFKYILPLSHRSFLLLTCTIFTLLLTLIGVNINILLPIQKKVGYLIKDDSEKQATITNTKYSTL**ANPYISVANIMLQ**
NYVKQREKYNYDTLKEQFTFIKNASTSIVYMQFANFMNIDNSLSPVIRYQKLYRRSINIISINNINNNEATVTFESLAQNNTGEILENMLWEAKIGFIMDSI
STSTLHNMPFHFIVTSYKLKLLRNKNQQ



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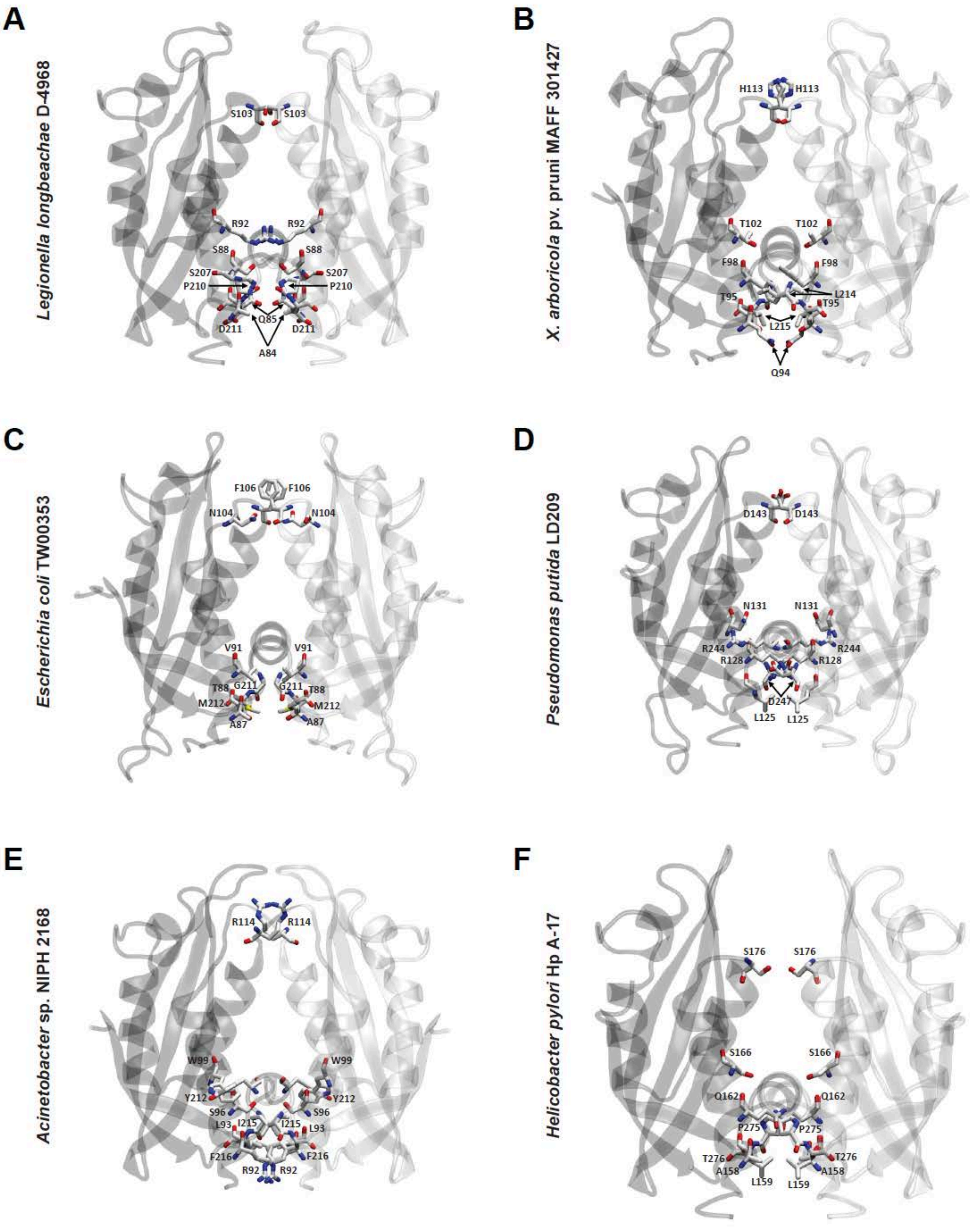

Fig. 3 (Document S4)

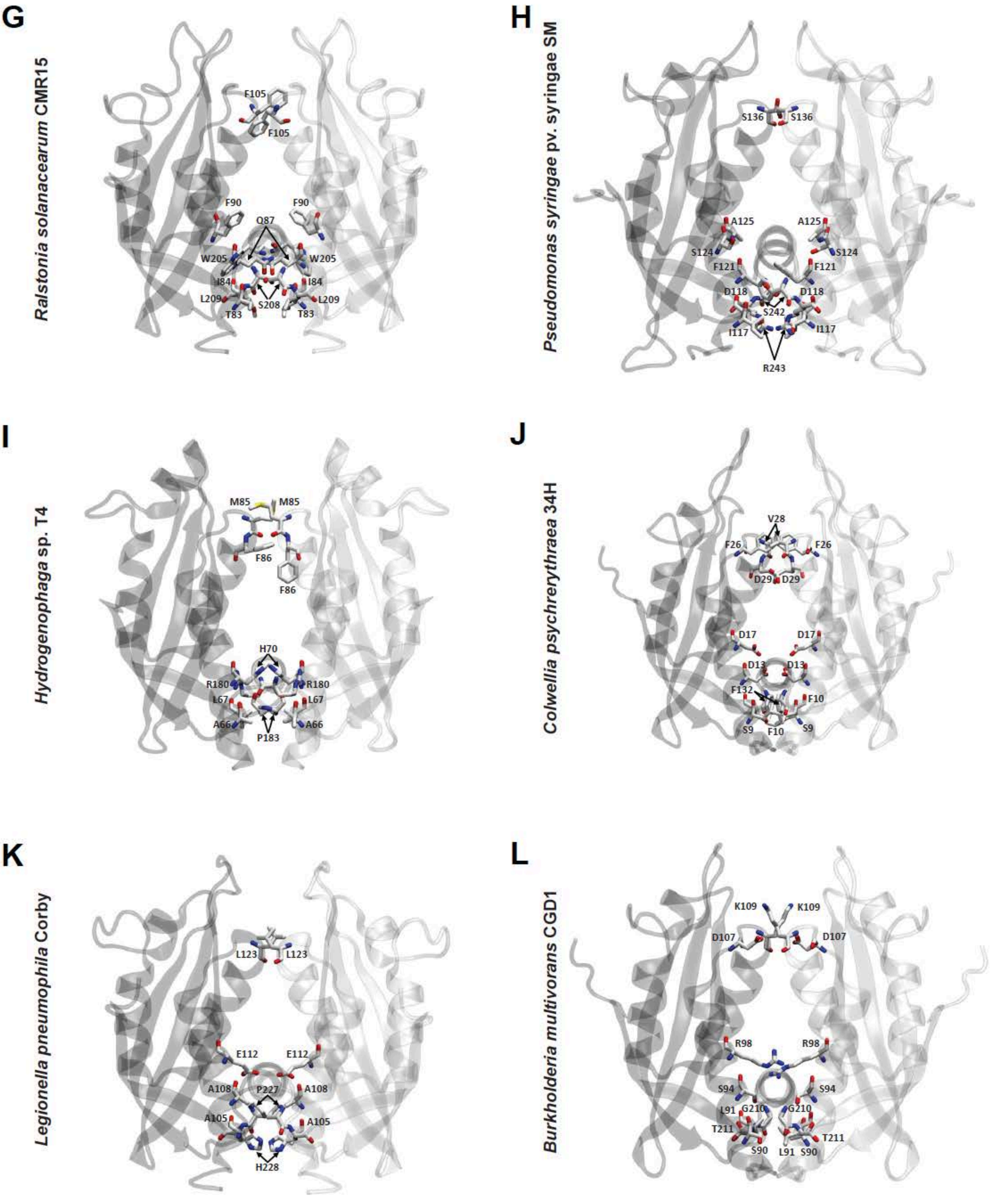

Fig. 3 (Document S4)

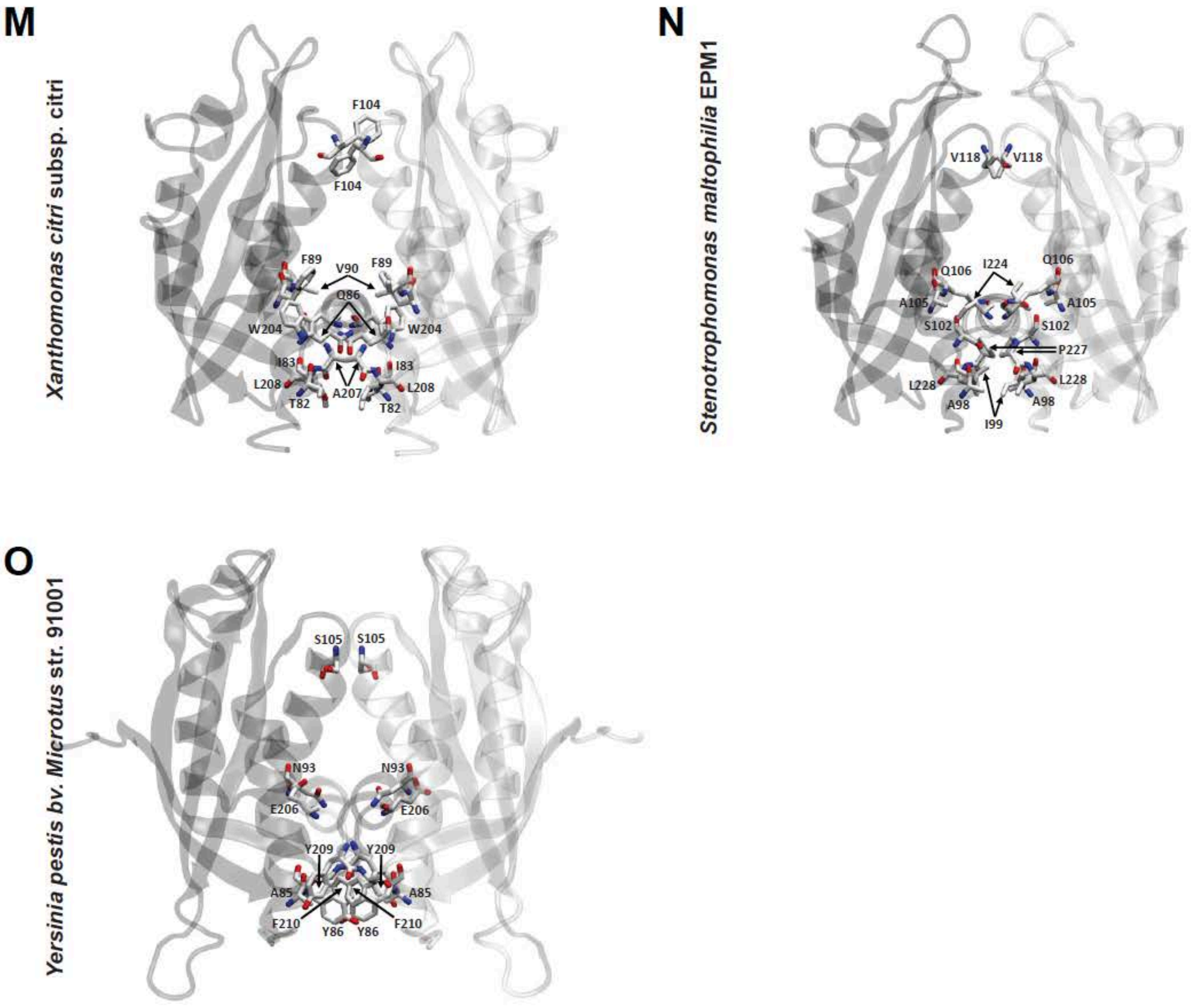

Fig. 3 (Document S4)