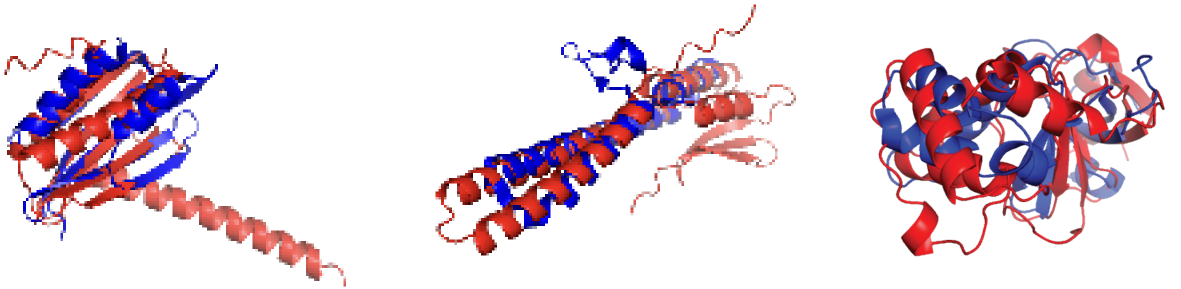

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TM-score=0.6648

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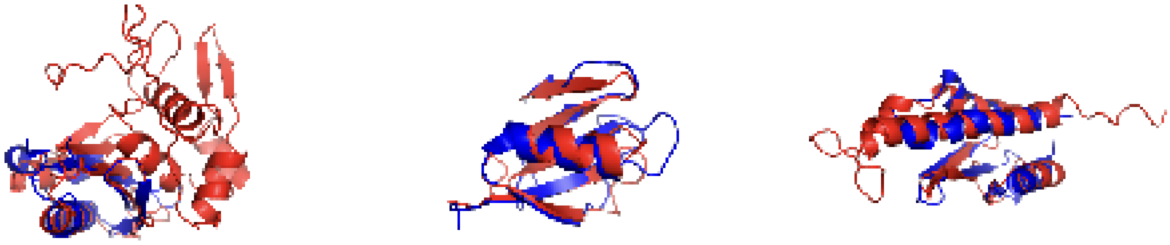

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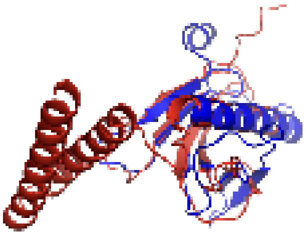
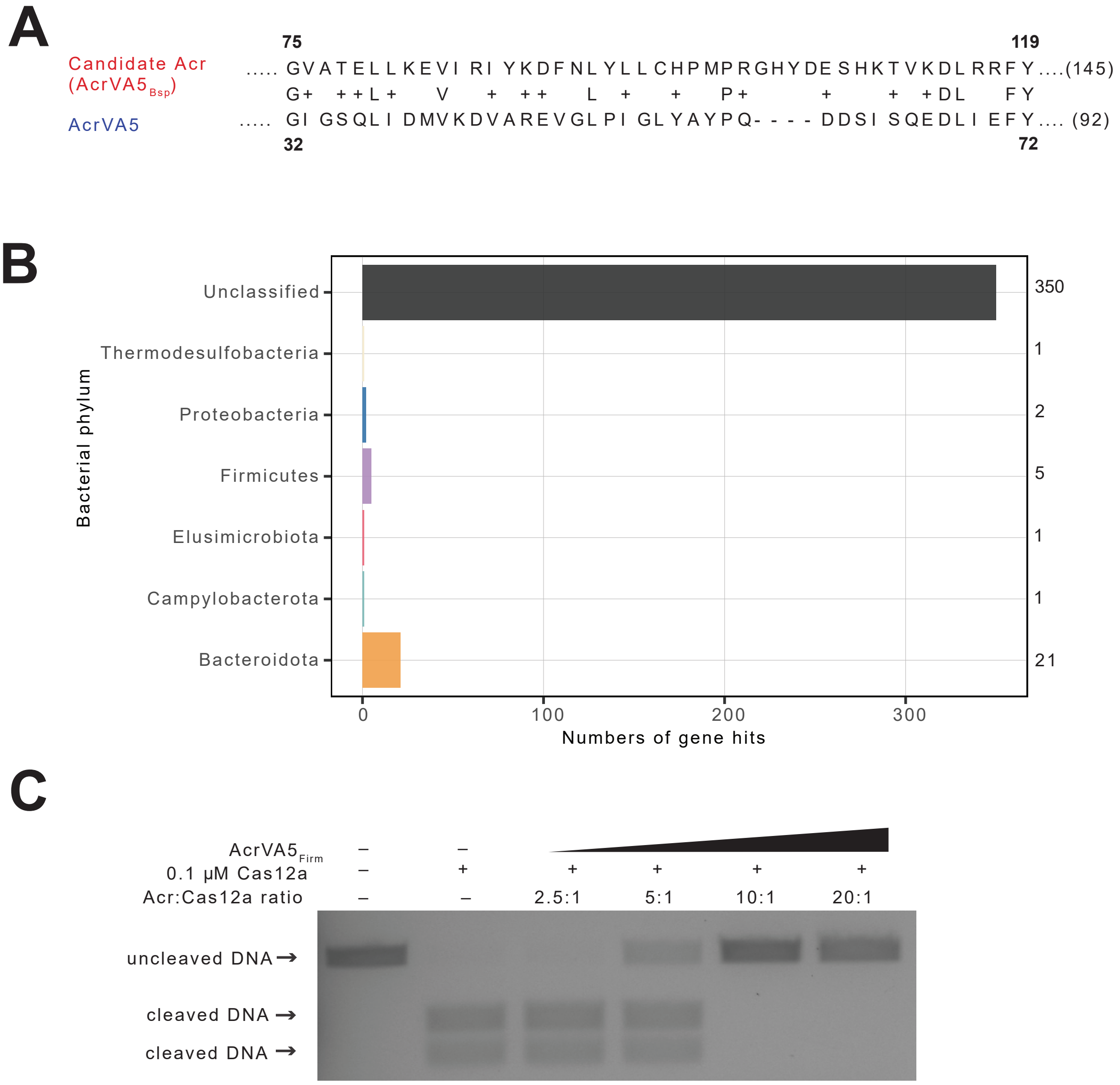
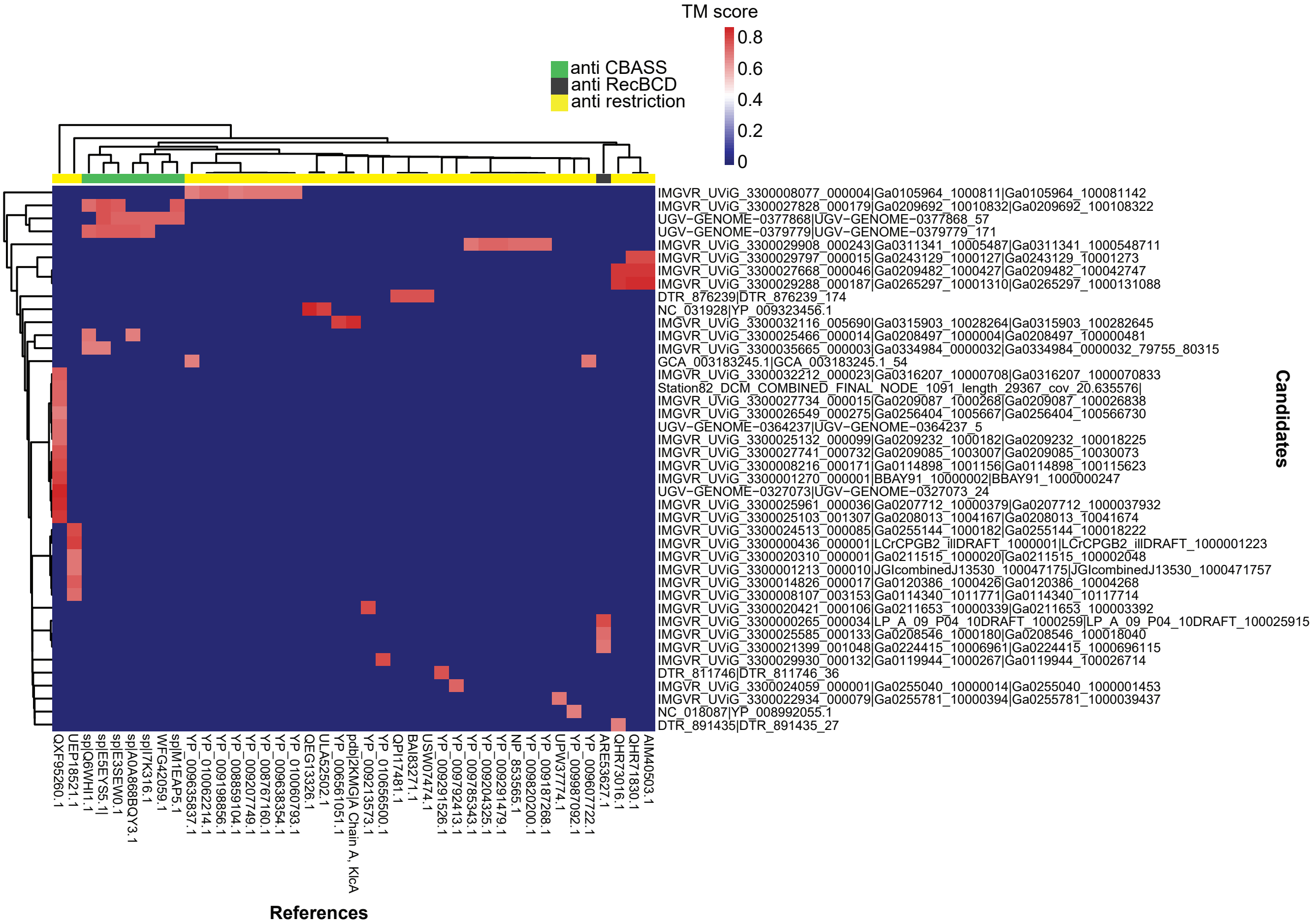


AcrIIC2
UGV-GENOME-0368711|UGV-GENOME-0368711_46
TM-score=0.6017

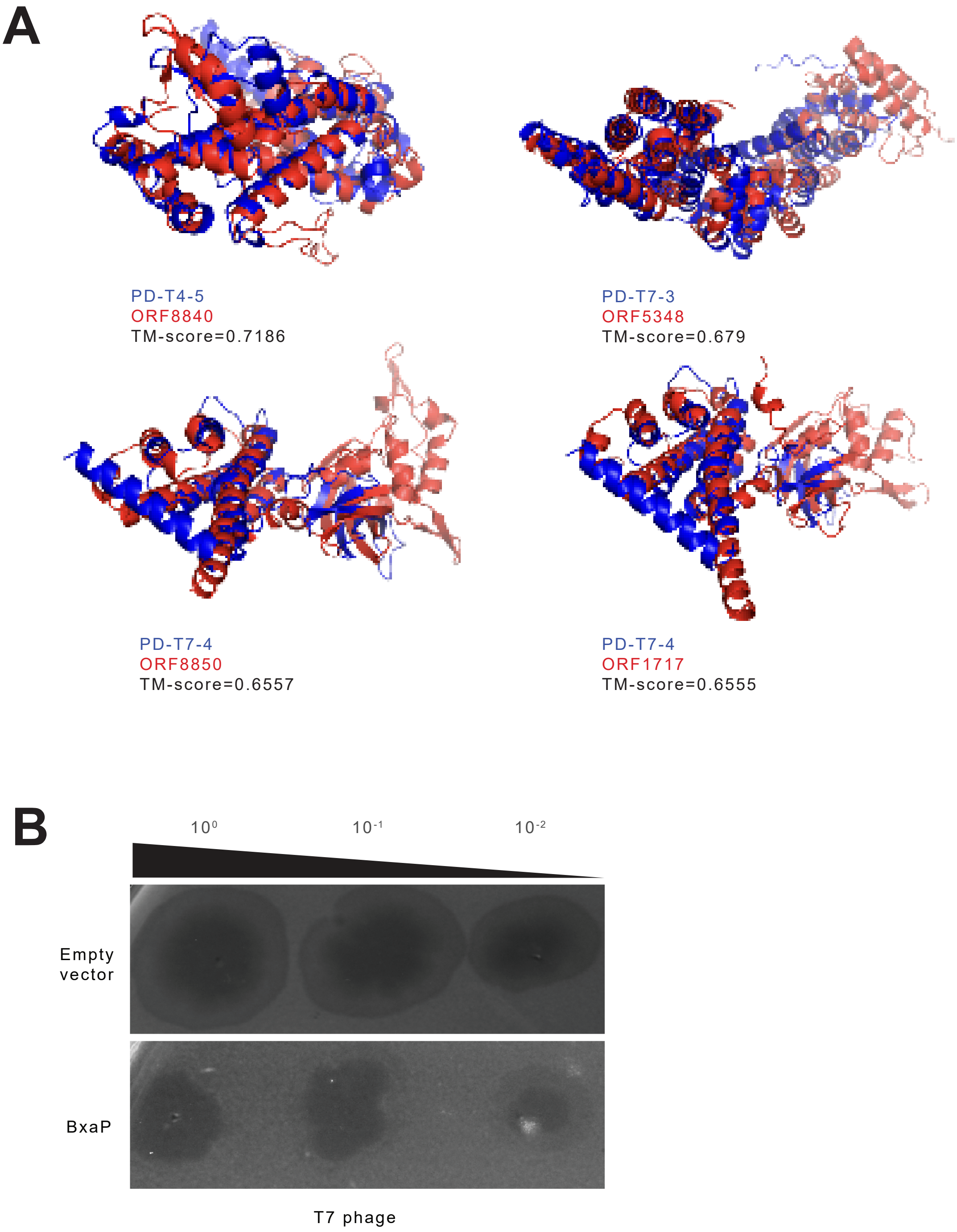
Supplementary Figure 1: Structural comparison between putative and known Acr proteins. AlphaFold2 model of candidate Acrs (in red) superposed to known Acr proteins (blue). The candidate Acrs are indicated by their IMG/VR gene id.



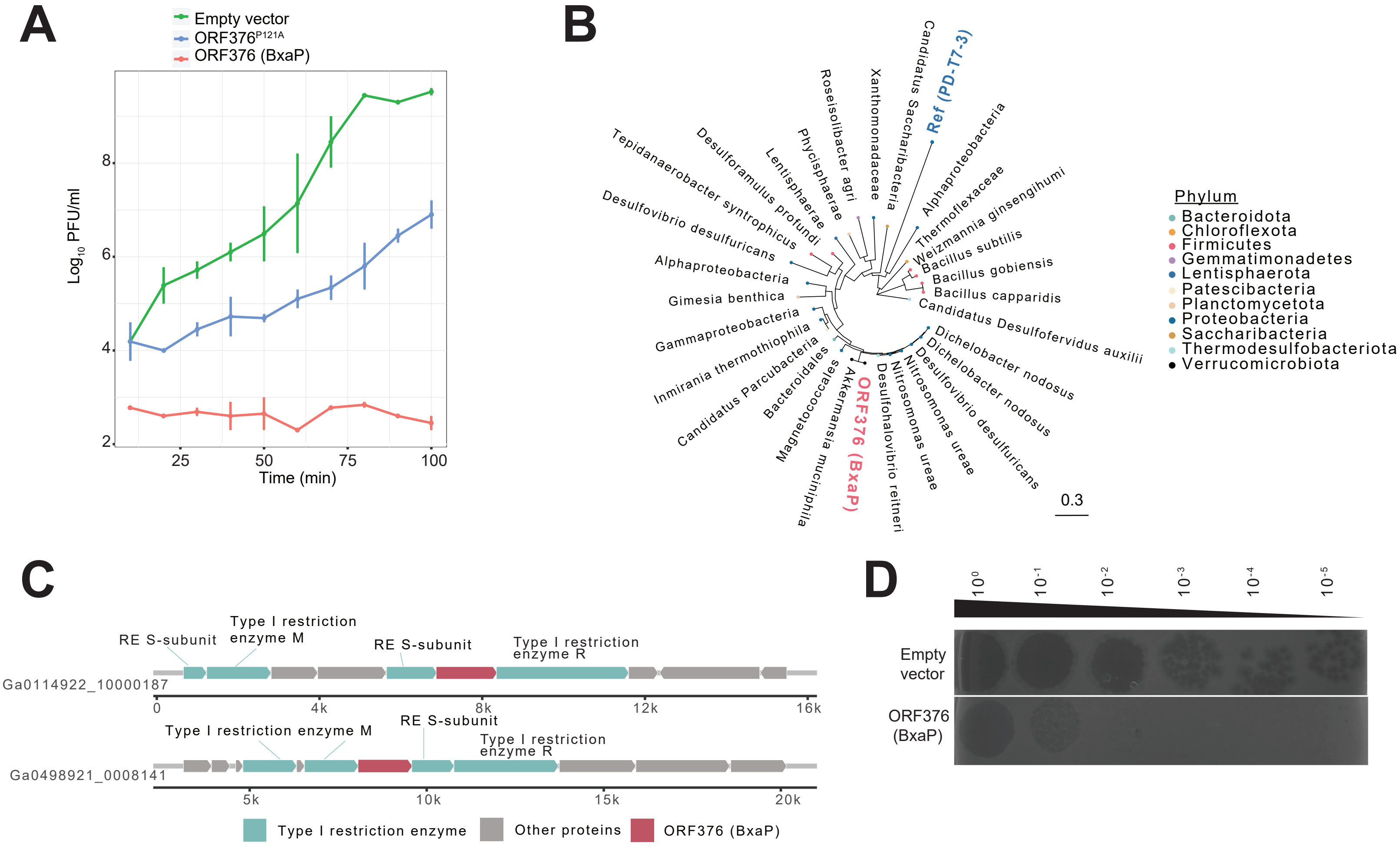
Supplementary Figure 2: Characterization of AcrVA5$_{Bsp}$ and homolog from a Firmicutes host. (A) Primary sequence alignment between AcrVA5 and AcrVA5$_{Bsp}$. The numbers in parenthesis show the protein length, while the numbers in bold represent the alignment start and end positions. No significant similarity between both proteins at an evalue threshold of 0.05. (B) Distribution of BLAST hits of protein-coding genes in the scaffold containing AcrVA5$_{Bsp}$. The BLAST search was implemented in the online server of IMG/VR. Though most of the genes were unassigned, 21 genes had hits in a *Bacteroidota* genome. (C) Agarose gel showing LbCas12a-mediated dsDNA cleavage in the presence of increasing amounts of AcrVA5$_{Firm}$. Acr concentrations used are 0.25, 0.5, 1, and 2 μM. Image is representative of duplicate. Source data are provided as a Source Data file.



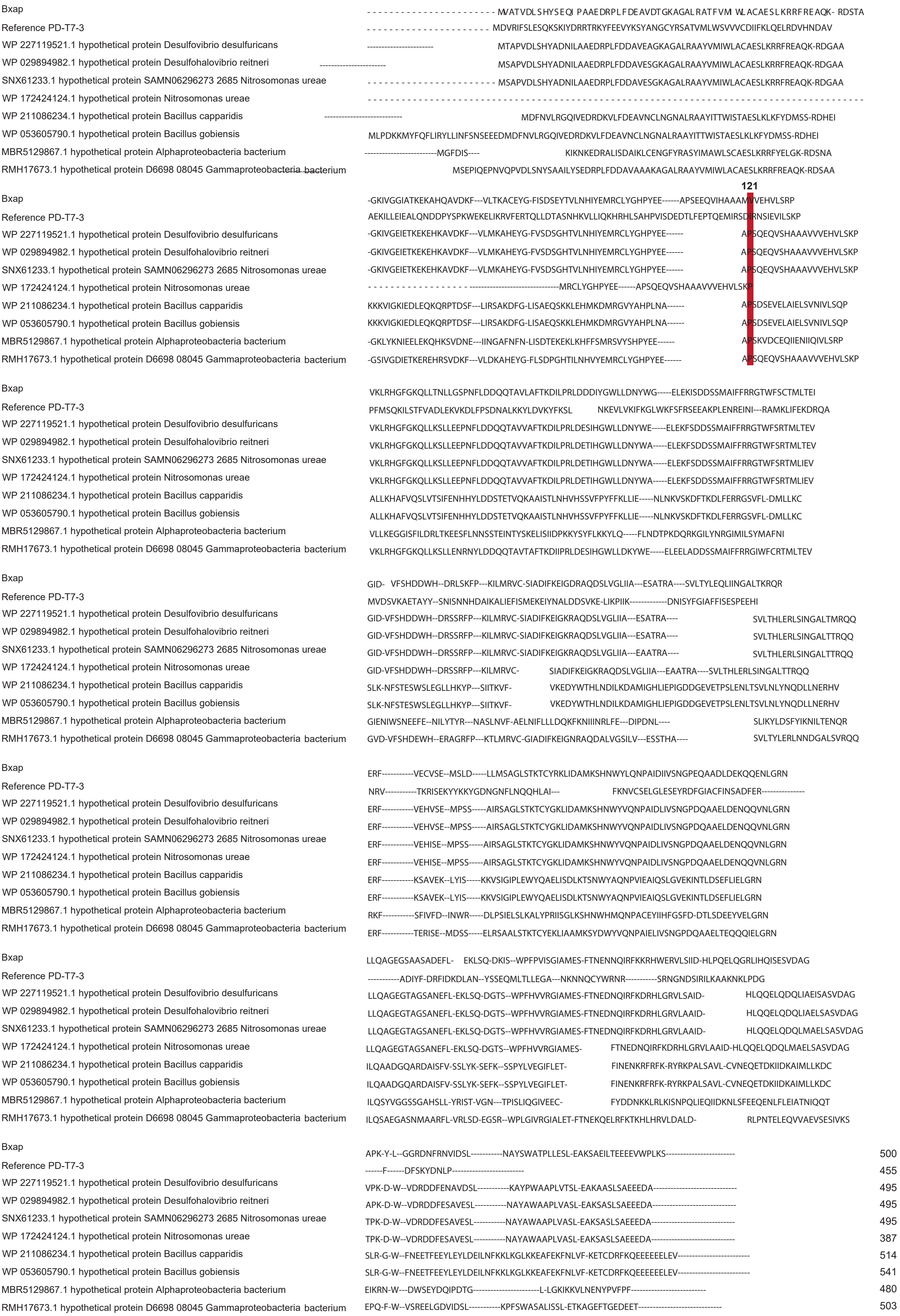
Supplementary Figure 3: Identification of remote homologs of other phage counter-defenses. Heatmap showing proteins in IMG/VR (rows) with no sequence similarity, but having high structural similarities to known counter-defenses (columns). For instance, DTR_891435|DTR_891435_27 is structurally similar to a known anti-restriction protein (QHR73016.1)



Supplementary Figure 4: Structural similarities between putative and known defense proteins and effect of BxaP expression on T7 infection.
(A) AlphaFold2 model of candidate defense proteins (in red) superposed to predicted single-protein defense systems reported in Vassallo *et al.* (blue). (B) A 10-fold serial dilution plaque assay of T7 phage on *E. coli* strain containing either an empty vector or overexpressing BxaP. Image is representative of triplicate.



Supplementary Figure 5: BxaP protects against phage infection.
(A) One-step growth curves showing measurements of plaque forming units (PFU) over time in T4-infected *E. coli* strain containing either an empty vector, or overexpressing WT or mutant BxaP. Experiments were conducted in duplicates, and error bars represent SEM. (B) Phylogenetic distribution of BxaP homologs in NCBI non-redundant database. Branch tips are colored based on bacteria phyla. PD-T7-3 is included in the tree for reference purposes. (C) Examples of phage genomic loci containing BxaP. The IMG/VR ID are shown beside each scaffold. (D) A 10-fold serial dilution plaque assay of T4 phage on an *E. coli* strain (DH10B) lacking both BREX and type-I RM systems. Image is representative of duplicate. Source data are provided as a Source Data file.



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Bxap                                                                  -----------------------MVATVDLSHYSEQIPAAEDRPLFDEAVDTGKAGALRATFVMIWLACAESLKRRFREAQK-RDSTA
Reference PD-T7-3                                                     ----------------------MDVRIFSLESQKSKIYDRRTRKYFEEVYKSYANGCYRSATVMLWSVVVCDIIFKLQELRDVHNDAV
WP 227119521.1 hypothetical protein Desulfovibrio desulfuricans       -----------------------       MTAPVDLSHYADNILAAEDRPLFDDAVEAGKAGALRAAYVMIWLACAESLKRRFREAQK-RDGAA
WP 029894982.1 hypothetical protein Desulfohalovibrio reitneri        -----------------------       MSAPVDLSHYADNILAAEDRPLFDDAVESGKAGALRAAYVMIWLACAESLKRRFREAQK-RDGAA
SNX61233.1 hypothetical protein SAMN06296273 2685 Nitrosomonas ureae -----------------------MSAPVDLSHYADNILAAEDRPLFDDAVESGKAGALRAAYVMIWLACAESLKRRFREAQK-RDGAA
WP 172424124.1 hypothetical protein Nitrosomonas ureae                ---------------------------------------------------------------------------------------------
WP 211086234.1 hypothetical protein Bacillus capparidis               ---------------------------          MDFNVLRGQIVEDRDKVLFDEAVNCLNGNALRAAYITTWISTAESLKLKFYDMSS-RDHEI
WP 053605790.1 hypothetical protein Bacillus gobiensis                MLPDKKMYFQFLIRYLLINFSNSEEEDMDFNVLRGQIVEDRDKVLFDEAVNCLNGNALRAAYITTWISTAESLKLKFYDMSS-RDHEI
MBR5129867.1 hypothetical protein Alphaproteobacteria bacterium      -------------------------MGFDIS----          KIKNKEDRALISDAIKLCENGFYRASYIMAWLSCAESLKRRFYELGK-RDSNA
RMH17673.1 hypothetical protein D6698 08045 Gammaproteobacteria bacterium      MSEPIQEPNVQPVDLSNYSAAILYSEDRPLFDDAVAAAKAGALRAAYVMIWLACAESLKRRFREAQK-RDSAA

                                                                                                                                                  121
Bxap                                                                  -GKIVGGIATKEKAHQAVDKF---VLTKACEYG-FISDSEYTVLNHIYEMRCLYGHPYEE------APSEEQVIHAAAMVVEHVLSRP
Reference PD-T7-3                                                     AEKILLEIEALQNDDPYSPKWEKELIKRVFERTQLLDTASNHKVLLIQKHRHLSAHPVISDEDTLFEPTQEMIRSDIRNSIEVILSKP
WP 227119521.1 hypothetical protein Desulfovibrio desulfuricans       -GKIVGEIETKEKEHKAVDKF---VLMKAHEYG-FVSDSGHTVLNHIYEMRCLYGHPYEE------          APSQEQVSHAAAVVVEHVLSKP
WP 029894982.1 hypothetical protein Desulfohalovibrio reitneri        -GKIVGEIETKEKEHKAVDKF---VLMKAHEYG-FVSDSGHTVLNHIYEMRCLYGHPYEE------          APSQEQVSHAAAVVVEHVLSKP
SNX61233.1 hypothetical protein SAMN06296273 2685 Nitrosomonas ureae -GKIVGEIETKEKEHKAVDKF---VLMKAHEYG-FVSDSGHTVLNHIYEMRCLYGHPYEE------          APSQEQVSHAAAVVVEHVLSKP
WP 172424124.1 hypothetical protein Nitrosomonas ureae                ------------------------------------------------MRCLYGHPYEE------APSQEQVSHAAAVVVEHVLSKP
WP 211086234.1 hypothetical protein Bacillus capparidis               KKKVIGKIEDLEQKQRPTDSF---LIRSAKDFG-LISAEQSKKLEHMKDMRGVYAHPLNA------          APSDSEVELAIELSVNIVLSQP
WP 053605790.1 hypothetical protein Bacillus gobiensis                KKKVIGKIEDLEQKQRPTDSF---LIRSAKDFG-LISAEQSKKLEHMKDMRGVYAHPLNA------          APSDSEVELAIELSVNIVLSQP
MBR5129867.1 hypothetical protein Alphaproteobacteria bacterium      -GKLYKNIEELEKQHKSVDNE---IINGAFNFN-LISDTEKEKLKHFFSMRSVYSHPYEE------          APSKVDCEQIIENIIQIVLSRP
RMH17673.1 hypothetical protein D6698 08045 Gammaproteobacteria bacterium -GSIVGDIETKEREHRSVDKF---VLDKAHEYG-FLSDPGHTILNHVYEMRCLYGHPYEE------          APSQEQVSHAAAVVVEHVLSKP

Bxap                                                                  VKLRHGFGKQLLTNLLGSPNFLDDQQTAVLAFTKDILPRLDDDIYGWLLDNYWG-----ELEKISDDSSMAIFFRRGTWFSCTMLTEI
Reference PD-T7-3                                                     PFMSQKILSTFVADLEKVKDLFPSDNALKKYLDVKYFKSL        NKEVLVKIFKGLWKFSFRSEEAKPLENREINI---RAMKLIFEKDRQA
WP 227119521.1 hypothetical protein Desulfovibrio desulfuricans       VKLRHGFGKQLLKSLLEEPNFLDDQQTAVVAFTKDILPRLDESIHGWLLDNYWE-----ELEKFSDDSSMAIFFRRGTWFSRTMLTEV
WP 029894982.1 hypothetical protein Desulfohalovibrio reitneri        VKLRHGFGKQLLKSLLEEPNFLDDQQTAVVAFTKDILPRLDETIHGWLLDNYWA-----ELEKFSDDSSMAIFFRRGTWFSRTMLTEV
SNX61233.1 hypothetical protein SAMN06296273 2685 Nitrosomonas ureae VKLRHGFGKQLLKSLLEEPNFLDDQQTAVVAFTKDILPRLDETIHGWLLDNYWA-----ELEKFSDDSSMAIFFRRGTWFSRTMLIEV
WP 172424124.1 hypothetical protein Nitrosomonas ureae                VKLRHGFGKQLLKSLLEEPNFLDDQQTAVVAFTKDILPRLDETIHGWLLDNYWA-----ELEKFSDDSSMAIFFRRGTWFSRTMLIEV
WP 211086234.1 hypothetical protein Bacillus capparidis               ALLKHAFVQSLVTSIFENHHYLDDSTETVQKAAISTLNHVHSSVFPYFFKLLIE-----NLNKVSKDFTKDLFERRGSVFL-DMLLKC
WP 053605790.1 hypothetical protein Bacillus gobiensis                ALLKHAFVQSLVTSIFENHHYLDDSTETVQKAAISTLNHVHSSVFPYFFKLLIE-----NLNKVSKDFTKDLFERRGSVFL-DMLLKC
MBR5129867.1 hypothetical protein Alphaproteobacteria bacterium      VLLKEGGISFILDRLTKEESFLNNSSTEINTYSKELISIIDPKKYSYFLKKYLQ-----FLNDTPKDQRKGILYNRGIMILSYMAFNI
RMH17673.1 hypothetical protein D6698 08045 Gammaproteobacteria bacterium VKLRHGFGKQLLKSLLENRNYLDDQQTAVTAFTKDIIPRLDESIHGWLLDKYWE-----ELEELADDSSMAIFFRRGIWFCRTMLTEV

Bxap                                                                  GID- VFSHDDWH--DRLSKFP---KILMRVC-SIADIFKEIGDRAQDSLVGLIIA---ESATRA----SVLTYLEQLIINGALTKRQR
Reference PD-T7-3                                                     MVDSVKAETAYY--SNISNNHDAIKALIEFISMEKEIYNALDDSVKE-LIKPIIK------------DNISYFGIAFFISESPEEHI
WP 227119521.1 hypothetical protein Desulfovibrio desulfuricans       GID-VFSHDDWH--DRSSRFP---KILMRVC-SIADIFKEIGKRAQDSLVGLIIA---ESATRA----          SVLTHLERLSINGALTMRQQ
WP 029894982.1 hypothetical protein Desulfohalovibrio reitneri        GID-VFSHDDWH--DRSSRFP---KILMRVC-SIADIFKEIGKRAQDSLVGLIIA---ESATRA----          SVLTHLERLSINGALTTRQQ
SNX61233.1 hypothetical protein SAMN06296273 2685 Nitrosomonas ureae GID-VFSHDDWH--DRSSRFP---KILMRVC-SIADIFKEIGKRAQDSLVGLIIA---EAATRA----          SVLTHLERLSINGALTTRQQ
WP 172424124.1 hypothetical protein Nitrosomonas ureae                GID-VFSHDDWH--DRSSRFP---KILMRVC-          SIADIFKEIGKRAQDSLVGLIIA---EAATRA----SVLTHLERLSINGALTTRQQ
WP 211086234.1 hypothetical protein Bacillus capparidis               SLK-NFSTESWSLEGLLHKYP---SIITKVF-          VKEDYWTHLNDILKDAMIGHLIEPIGDDGEVETPSLENLTSVLNLYNQDLLNERHV
WP 053605790.1 hypothetical protein Bacillus gobiensis                SLK-NFSTESWSLEGLLHKYP---SIITKVF-          VKEDYWTHLNDILKDAMIGHLIEPIGDDGEVETPSLENLTSVLNLYNQDLLNERHV
MBR5129867.1 hypothetical protein Alphaproteobacteria bacterium      GIENIWSNEEFE--NILYTYR---NASLNVF-AELNIFLLLDQKFKNIIINRLFE---DIPDNL----          SLIKYLDSFYIKNILTENQR
RMH17673.1 hypothetical protein D6698 08045 Gammaproteobacteria bacterium GVD-VFSHDEWH--ERAGRFP---KTLMRVC-GIADIFKEIGNRAQDALVGSILV---ESSTHA----          SVLTYLERLNNDGALSVRQQ

Bxap                                                                  ERF-----------VECVSE--MSLD-----LLMSAGLSTKTCYRKLIDAMKSHNWYLQNPAIDIIVSNGPEQAADLDEKQQENLGRN
Reference PD-T7-3                                                     NRV-----------TKRISEKYYKKYGDNGNFLNQQHLAI---          FKNVCSELGLESEYRDFGIACFINSADFER---------------
WP 227119521.1 hypothetical protein Desulfovibrio desulfuricans       ERF-----------VEHVSE--MPSS-----AIRSAGLSTKTCYGKLIDAMKSHNWYVQNPAIDLIVSNGPDQAAELDENQQVNLGRN
WP 029894982.1 hypothetical protein Desulfohalovibrio reitneri        ERF-----------VEHVSE--MPSS-----AIRSAGLSTKTCYGKLIDAMKSHNWYVQNPAIDLIVSNGPDQAAELDENQQVNLGRN
SNX61233.1 hypothetical protein SAMN06296273 2685 Nitrosomonas ureae ERF-----------VEHISE--MPSS-----AIRSAGLSTKTCYGKLIDAMKSHNWYVQNPAIDLIVSNGPDQAAELDENQQVNLGRN
WP 172424124.1 hypothetical protein Nitrosomonas ureae                ERF-----------VEHISE--MPSS-----AIRSAGLSTKTCYGKLIDAMKSHNWYVQNPAIDLIVSNGPDQAAELDENQQVNLGRN
WP 211086234.1 hypothetical protein Bacillus capparidis               ERF-----------KSAVEK--LYIS-----KKVSIGIPLEWYQAELISDLKTSNWYAQNPVIEAIQSLGVEKINTLDSEFLIELGRN
WP 053605790.1 hypothetical protein Bacillus gobiensis                ERF-----------KSAVEK--LYIS-----KKVSIGIPLEWYQAELISDLKTSNWYAQNPVIEAIQSLGVEKINTLDSEFLIELGRN
MBR5129867.1 hypothetical protein Alphaproteobacteria bacterium      RKF-----------SFIVFD--INWR-----DLPSIELSLKALYPRIISGLKSHNWHMQNPACEYIIHFGSFD-DTLSDEEYVELGRN
RMH17673.1 hypothetical protein D6698 08045 Gammaproteobacteria bacterium ERF-----------TERISE--MDSS-----ELRSAALSTKTCYEKLIAAMKSYDWYVQNPAIELIVSNGPDQAAELTEQQQIELGRN

Bxap                                                                  LLQAGEGSAASADEFL-    EKLSQ-DKIS--WPFPVISGIAMES-FTNENNQIRFKKRHWERVLSIID-HLPQELQGRLIHQISESVDAG
Reference PD-T7-3                                                     -----------ADIYF-DRFIDKDLAN--YSSEQMLTLLEGA---NKNNQCYWRNR-----------SRNGNDSIRILKAAKNKLPDG
WP 227119521.1 hypothetical protein Desulfovibrio desulfuricans       LLQAGEGTAGSANEFL-EKLSQ-DGTS--WPFHVVRGIAMES-FTNEDNQIRFKDRHLGRVLSAID-          HLQQELQDQLIAEISASVDAG
WP 029894982.1 hypothetical protein Desulfohalovibrio reitneri        LLQAGEGTAGSANEFL-EKLSQ-DGTS--WPFHVVRGIAMES-FTNEDNQIRFKDRHLGRVLAAID-          HLQQELQDQLIAELSASVDAG
SNX61233.1 hypothetical protein SAMN06296273 2685 Nitrosomonas ureae LLQAGEGTAGSANEFL-EKLSQ-DGTS--WPFHVVRGIAMES-FTNEDNQIRFKDRHLGRVLAAID-          HLQQELQDQLMAELSASVDAG
WP 172424124.1 hypothetical protein Nitrosomonas ureae                LLQAGEGTAGSANEFL-EKLSQ-DGTS--WPFHVVRGIAMES-          FTNEDNQIRFKDRHLGRVLAAID-HLQQELQDQLMAELSASVDAG
WP 211086234.1 hypothetical protein Bacillus capparidis               ILQAADGQARDAISFV-SSLYK-SEFK--SSPYLVEGIFLET-          FINENKRFRFK-RYRKPALSAVL-CVNEQETDKIIDKAIMLLKDC
WP 053605790.1 hypothetical protein Bacillus gobiensis                ILQAADGQARDAISFV-SSLYK-SEFK--SSPYLVEGIFLET-          FINENKRFRFK-RYRKPALSAVL-CVNEQETDKIIDKAIMLLKDC
MBR5129867.1 hypothetical protein Alphaproteobacteria bacterium      ILQSYVGGSSGAHSLL-YRIST-VGN---TPISLIQGIVEEC-          FYDDNKKLRLKISNPQLIEQIIDKNLSFEEQENLFLEIATNIQQT
RMH17673.1 hypothetical protein D6698 08045 Gammaproteobacteria bacterium ILQSAEGASNMAARFL-VRLSD-EGSR--WPLGIVRGIALET-FTNEKQELRFKTKHLHRVLDALD-          RLPNTELEQVVAEVSESIVKS

Bxap                                                                  APK-Y-L--GGRDNFRNVIDSL-----------NAYSWATPLLESL-EAKSAEILTEEEEVWPLKS-----------------------    500
Reference PD-T7-3                                                     ------F------DFSKYDNLP------------------------                                                455
WP 227119521.1 hypothetical protein Desulfovibrio desulfuricans       VPK-D-W--VDRDDFENAVDSL-----------KAYPWAAPLVTSL-EAKAASLSAEEEDA----------------------------    495
WP 029894982.1 hypothetical protein Desulfohalovibrio reitneri        APK-D-W--VDRDDFESAVESL-----------NAYAWAAPLVASL-EAKSASLSAEEEDA----------------------------    495
SNX61233.1 hypothetical protein SAMN06296273 2685 Nitrosomonas ureae TPK-D-W--VDRDDFESAVESL-----------NAYAWAAPLVASL-EAKSASLSAEEEDA----------------------------    495
WP 172424124.1 hypothetical protein Nitrosomonas ureae                TPK-D-W--VDRDDFESAVESL-----------NAYAWAAPLVASL-EAKSASLSAEEEDA----------------------------    387
WP 211086234.1 hypothetical protein Bacillus capparidis               SLR-G-W--FNEETFEEYLEYLDEILNFKKLKGLKKEAFEKFNLVF-KETCDRFKQEEEEEELEV------------------------    514
WP 053605790.1 hypothetical protein Bacillus gobiensis                SLR-G-W--FNEETFEEYLEYLDEILNFKKLKGLKKEAFEKFNLVF-KETCDRFKQEEEEEELEV------------------------    541
MBR5129867.1 hypothetical protein Alphaproteobacteria bacterium      EIKRN-W---DWSEYDQIPDTG----------------------L-LGKIKKVLNENYPVFPF-------------------------    480
RMH17673.1 hypothetical protein D6698 08045 Gammaproteobacteria bacterium EPQ-F-W--VSREELGDVIDSL-----------KPFSWASALISSL-ETKAGEFTGEDEET----------------------------    503
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Supplementary Figure 6: Primary sequence alignment of some BxaP homologs. The shaded area shows mutated site used to generate the P121A BxaP mutant.