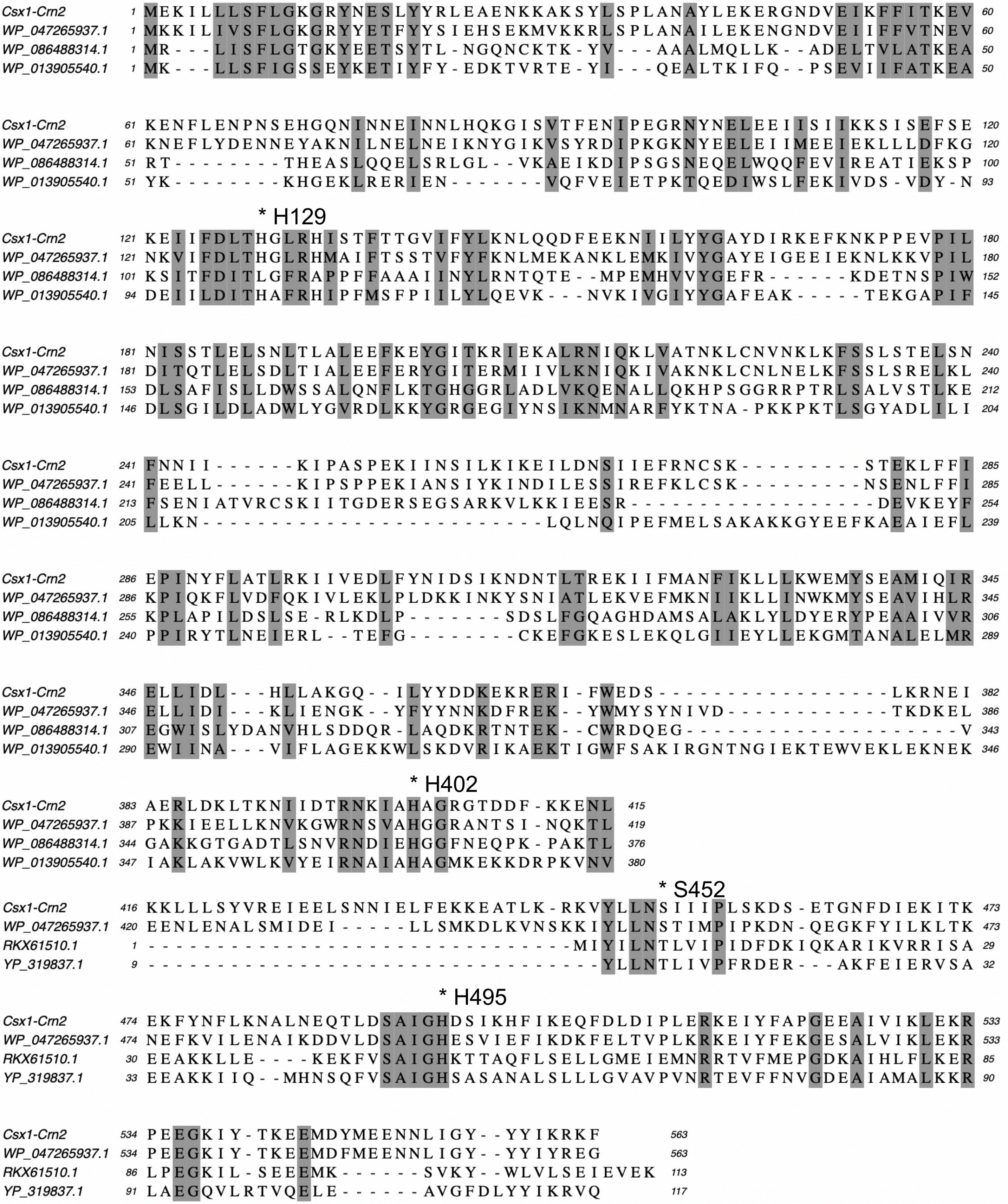

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Csx1-Crn2        1 MEKILLLSFLGKGRYNESLYYRLEAENKKAKSYLSPLANAYLEKERGNDVEIKFFITKEV 60
WP_047265937.1   1 MKKILIVSFLGKGRYYETFYYSIEHSEKMVKKRLSPLANAILEKENGNDVEIIFFVTNEV 60
WP_086488314.1   1 MR---LISFLGTGKYEETSYTL-NGQNCKTK-YV---AAALMQLLK--ADELTVLATKEA 50
WP_013905540.1   1 MK---LLSFIGSSEYKETIYFY-EDKTVRTE-YI---QEALTKIFQ--PSEVIIFATKEA 50

Csx1-Crn2       61 KENFLENPNSEHGQNINNEINNLHQKGISVTFENIPEGRNYNELEEIISIIKKSISEFSE 120
WP_047265937.1  61 KNEFLYDENNEYAKNILNELNEIKNYGIKVSYRDIPKGKNYEELEIIMEEIEKLLLDFKG 120
WP_086488314.1  51 RT--------THEASLQQELSRLGL--VKAEIKDIPSGSNEQELWQQFEVIREATIEKSP 100
WP_013905540.1  51 YK--------KHGEKLRERIEN-------VQFVEIETPKTQEDIWSLFEKIVDS-VDY-N 93

                           * H129
Csx1-Crn2      121 KEIIFDLTHGLRHISTFTTGVIFYLKNLQQDFEEKNIILYYGAYDIRKEFKNKPPEVPIL 180
WP_047265937.1 121 NKVIFDLTHGLRHMAIFTSSTVFYFKNLMEKANKLEMKIVYGAYEIGEEIEKNLKKVPIL 180
WP_086488314.1 101 KSITFDITLGFRAPPFFAAAIINYLRNTQTE--MPEMHVVYGEFR------KDETNSPIW 152
WP_013905540.1  94 DEIILDITHAFRHIPFMSFPIILYLQEVK---NVKIVGIYYGAFEAK-----TEKGAPIF 145

Csx1-Crn2      181 NISSTLELSNLTLALEEFKEYGITKRIEKALRNIQKLVATNKLCNVNKLKFSSLSTELSN 240
WP_047265937.1 181 DITQTLELSDLTIALEEFERYGITERMIIVLKNIQKIVAKNKLCNLNELKFSSLSRELKL 240
WP_086488314.1 153 DLSAFISLLDWSSALQNFLKTGHGGRLADLVKQENALLQKHPSGGRRPTRLSALVSTLKE 212
WP_013905540.1 146 DLSGILDLADWLYGVRDLKKYGRGEGIYNSIKNMNARFYKTNA-PKKPKTLSGYADLILI 204

Csx1-Crn2      241 FNNII------KIPASPEKIINSILKIKEILDNSIIEFRNCSK---------STEKLFFI 285
WP_047265937.1 241 FEELL------KIPSPPEKIANSIYKINDILESSIREFKLCSK---------NSENLFFI 285
WP_086488314.1 213 FSENIATVRCSKIITGDERSEGSARKVLKKIEESR------------------DEVKEYF 254
WP_013905540.1 205 LLKN-------------------------LQLNQIPEFMELSAKAKKGYEEFKAEAIEFL 239

Csx1-Crn2      286 EPINYFLATLRKIIVEDLFYNIDSIKNDNTLTREKIIFMANFIKLLLKWEMYSEAMIQIR 345
WP_047265937.1 286 KPIQKFLVDFQKIVLEKLPLDKKINKYSNIATLEKVEFMKNIIKLLINWKMYSEAVIHLR 345
WP_086488314.1 255 KPLAPILDSLSE-RLKDLP-------SDSLFGQAGHDAMSALAKLYLDYERYPEAAIVVR 306
WP_013905540.1 240 PPIRYTLNEIERL--TEFG--------CKEFGKESLEKQLGIIEYLLEKGMTANALELMR 289

Csx1-Crn2      346 ELLIDL---HLLAKGQ--ILYYDDKEKRERI-FWEDS-----------------LKRNEI 382
WP_047265937.1 346 ELLIDI---KLIENGK--YFYYNNKDFREK--YWMYSYNIVD------------TKDKEL 386
WP_086488314.1 307 EGWISLYDANVHLSDDQR-LAQDKRTNTEK--CWRDQEG--------------------V 343
WP_013905540.1 290 EWIINA---VIFLAGEKKWLSKDVRIKAEKTIGWFSAKIRGNTNGIEKTEWVEKLEKNEK 346

                                      * H402
Csx1-Crn2      383 AERLDKLTKNIIDTRNKIAHAGRGTDDF-KKENL 415
WP_047265937.1 387 PKKIEELLKNVKGWRNSVAHGGRANTSI-NQKTL 419
WP_086488314.1 344 GAKKGTGADTLSNVRNDIEHGGFNEQPK-PAKTL 376
WP_013905540.1 347 IAKLAKVWLKVYEIRNAIAHAGMKEKKDRPKVNV 380

                                                       * S452
Csx1-Crn2      416 KKLLLSYVREIEELSNNIELFEKKEATLK-RKVYLLNSIIIPLSKDS-ETGNFDIEKITK 473
WP_047265937.1 420 EENLENALSMIDEI-----LLSMKDLKVNSKKIYLLNSTIMPIPKDN-QEGKFYILKLTK 473
RKX61510.1       1 -------------------------------MIYILNTLVIPIDFDKIQKARIKVRRISA 29
YP_319837.1      9 ---------------------------------YLLNTLIVPFRDER---AKFEIERVSA 32

                                        * H495
Csx1-Crn2      474 EKFYNFLKNALNEQTLDSAIGHDSIKHFIKEQFDLDIPLERKEIYFAPGEEAIVIKLEKR 533
WP_047265937.1 474 NEFKVILENAIKDDVLDSAIGHESVIEFIKDKFELTVPLKRKEIYFEKGESALVIKLEKR 533
RKX61510.1      30 EEAKKLLE----KEKFVSAIGHKTTAQFLSELLGMEIEMNRRTVFMEPGDKAIHLFLKER 85
YP_319837.1     33 EEAKKIIQ--MHNSQFVSAIGHSASANALSLLLGVAVPVNRTEVFFNVGDEAIAMALKKR 90

Csx1-Crn2      534 PEEGKIY-TKEEMDYMEENNLIGY--YYIKRKF     563
WP_047265937.1 534 PEEGKIY-TKEEMDFMEENNLIGY--YYIYREG     563
RKX61510.1      86 LPEGKIL-SEEEMK------SVKY--WLVLSEIEVEK 113
YP_319837.1     91 LAEGQVLRTVQELE------AVGFDLYYIKRVQ     117
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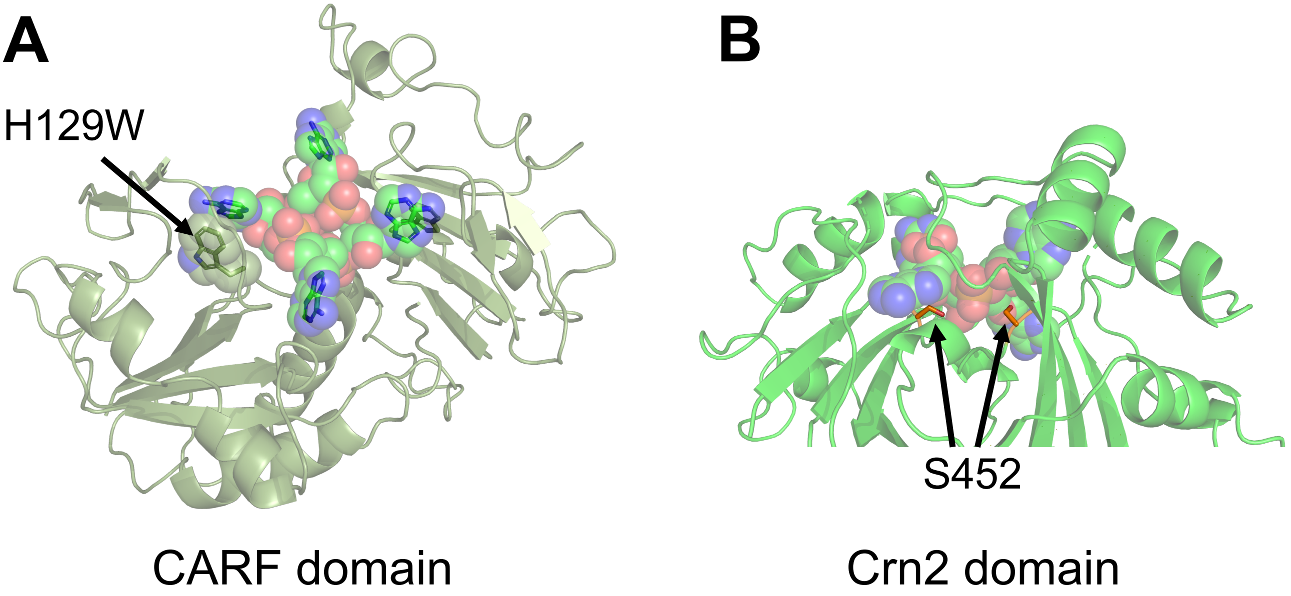
Supplementary figure 1. Multiple sequence alignment of Csx1-Crn2 with selected homologues. *M. piezophila* Csx1-Crn2 is aligned with the sole bifunctional homologue in NCBI, WP_047265937.1 from a related strain; with Csx1 homologues from *Thioflexothrix psekupsii* (WP_086488314.1) and *Pyrococcus yayanosii* (WP_013905540.1); and with Crn2 homologues from *Thermodesulfobacteria* bacterium (RKX61510) and Acidianus two-tailed virus (YP_319837.1). Residues mutated in this study are indicated.



CARF domain

Crn2 domain

Supplementary figure 2. Mutations designed to abolish cA4 binding in the CARF and Crn2 domains. A The CARF domain of the Csx1-Crn2 structure model is shown with cA_4 bound, based on the structure of Csx1 from *S. islandicus* (PDB 6R7B). Mutation of H129 to a tryptophan is predicted to result in a steric clash with the adenine base of cA4. **B** The Crn2 domain of the Csx1-Crn2 structure model is shown with cA_4 bound, based on the structure of AcrIII-1 from SIRV1 (PDB 6SCF). S452 is conserved in the Crn2 family and mutation to a bulkier tyrosine residue is predicted to block cA_4 binding. Colouring is consistent with that used in Figure 1.