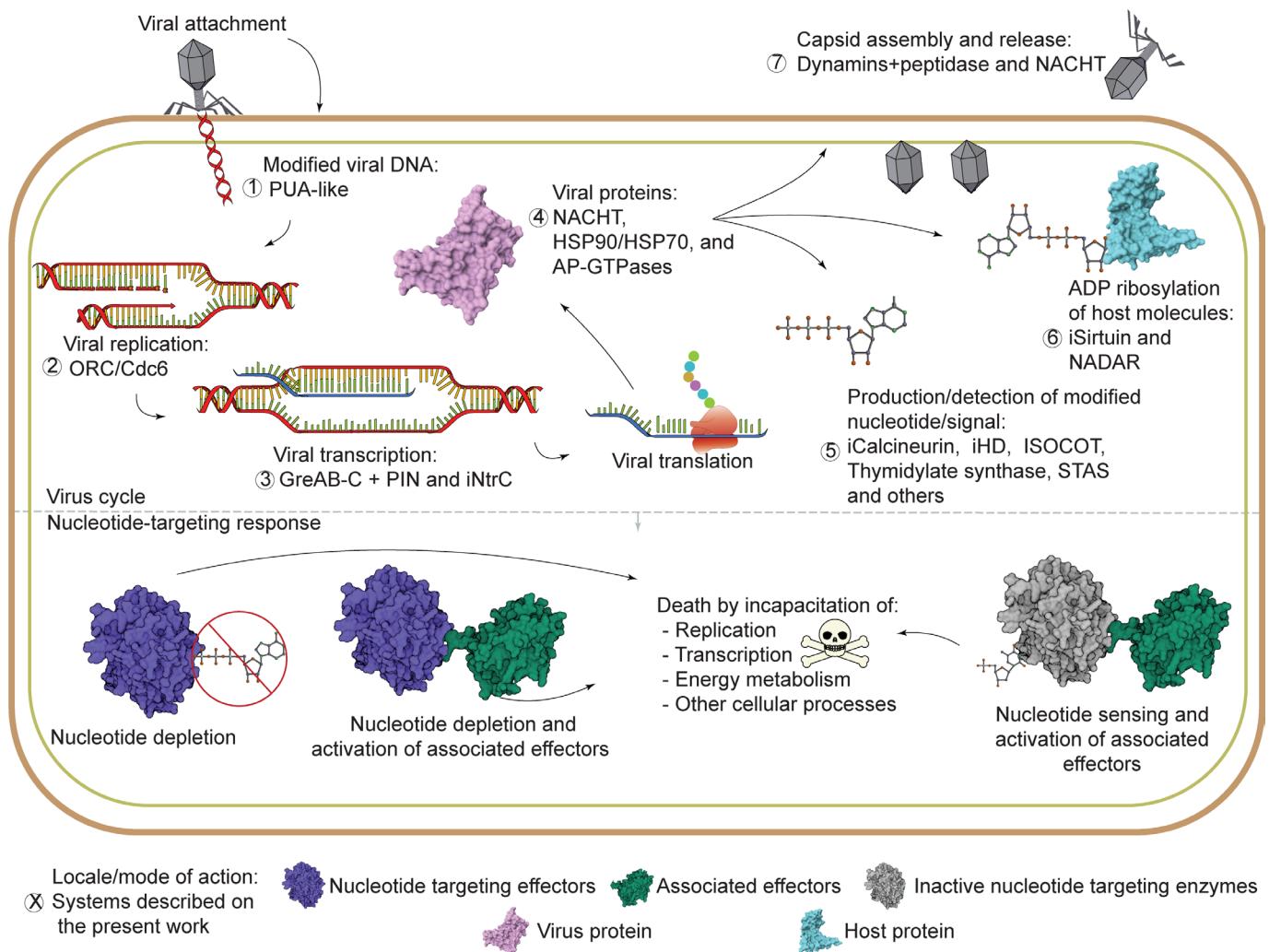


Functionally comparable but evolutionarily distinct nucleotide-targeting effectors help identify conserved paradigms across diverse immune systems

SUPPLEMENTARY MATERIAL

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National Institutes of Health, Bethesda, MD 20894



You can also access this content through a dedicated website, which can be found at:
<https://aravindlab.github.io/NTdegrade/>

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II. Supplementary Data

A. Genome contextual data

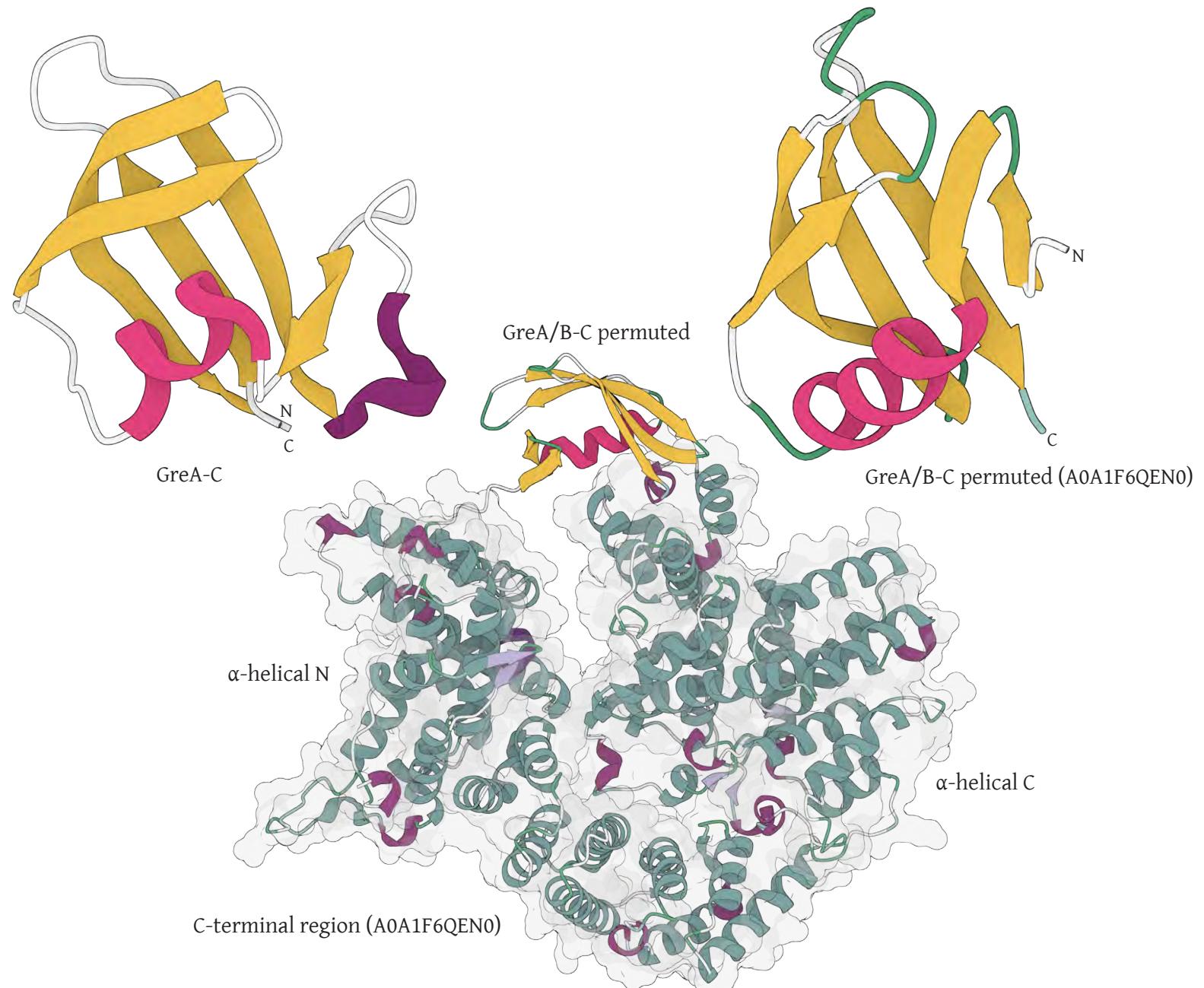
- 1. AEP-1D
- 2. AP-ATPase
- 3. AP-GTPase
- 4. Dynamin
- 5. GreAB-C
- 6. iHD
- 7. HSP70
- 8. HSP90
- 9. ISOCOT
- 10. MNS
- 11. NACHT
- 12. Nadar
- 13. ORC-Cdc6(Calcineurin-CE)
- 14. ORC-Cdc6(HD-CE)
- 15. ORC-Cdc6 (PRTase-CE)
- 16. ORC-Cdc6 (TIR)
- 17. ORC-Cdc6(SIR2)
- 18. ORC-Cdc6(Kinases)
- 19. ORC-Cdc6(Others)
- 20. PUA-like
- 21. ParB-CE
- 22. ParB
- 23. Pepco
- 24. PolBetaCCAA-like
- 25. RVT
- 26. SAMHD1
- 27. nSTAND4
- 28. nSTAND5
- 29. iSTAND3
- 30. iSirtuin
- 31. STAS big protein
- 32. STAS
- 33. Thymidylate synthase
- 34. iThymidylate synthase

B. Multiple sequence alignments

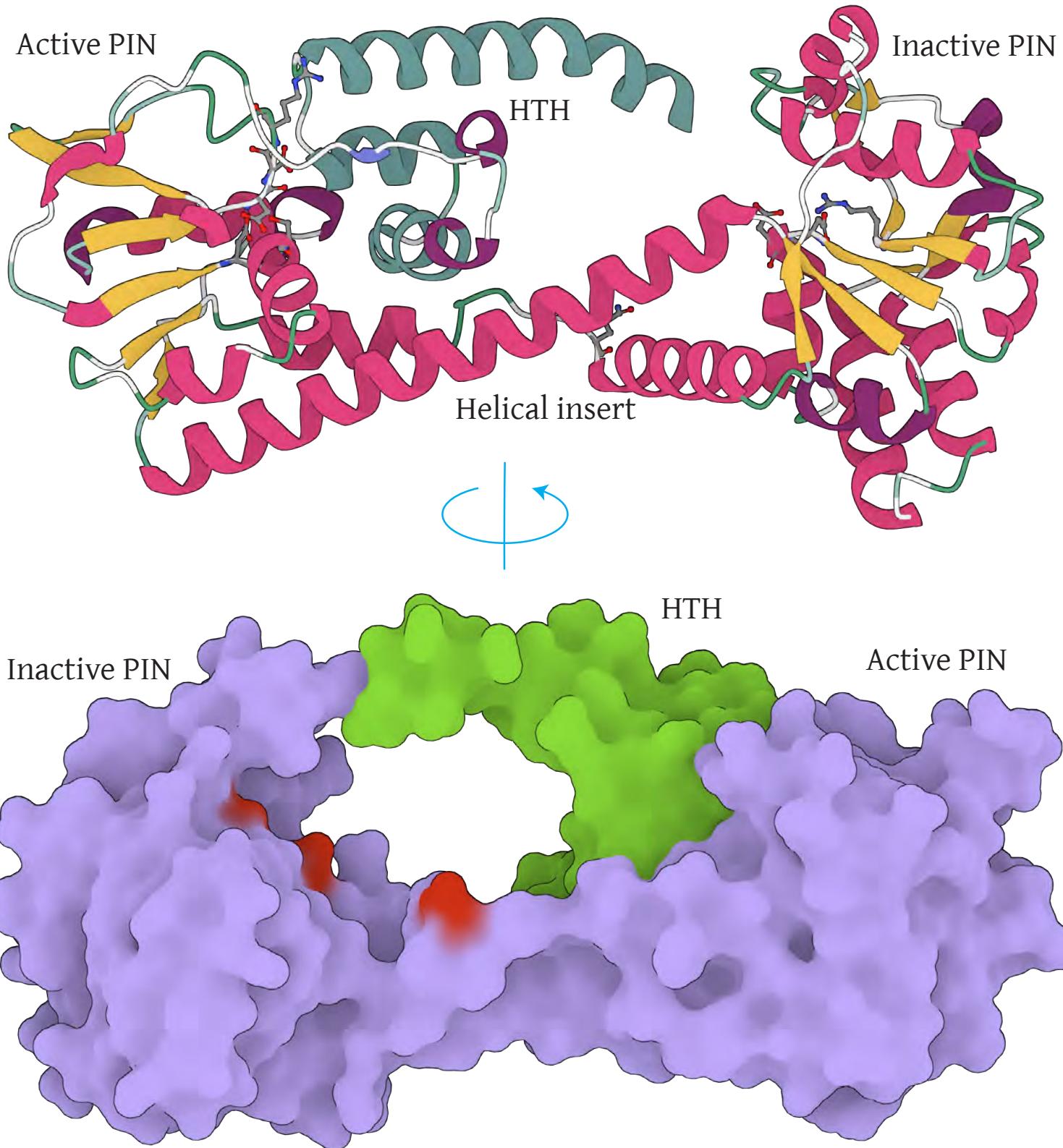
- 1. Calcineurin-CE
- 2. HD-CE
- 3. PRTase-CE
- 4. PUA-like-CE
- 5. iSirtuin-CE
- 6. Calcineurin-CE+iThymidylate-synthase
- 7. AEP-1D
- 8. REC+Calcineurin-CE
- 9. STAS+HisKin
- 10. STAS+PRTase
- 11. STAS+HisKin+iPRTase+PRTase
- 12. Pepco
- 13. iCalcineurin-CE
- 14. iHD

C. Phylogenetic Tree

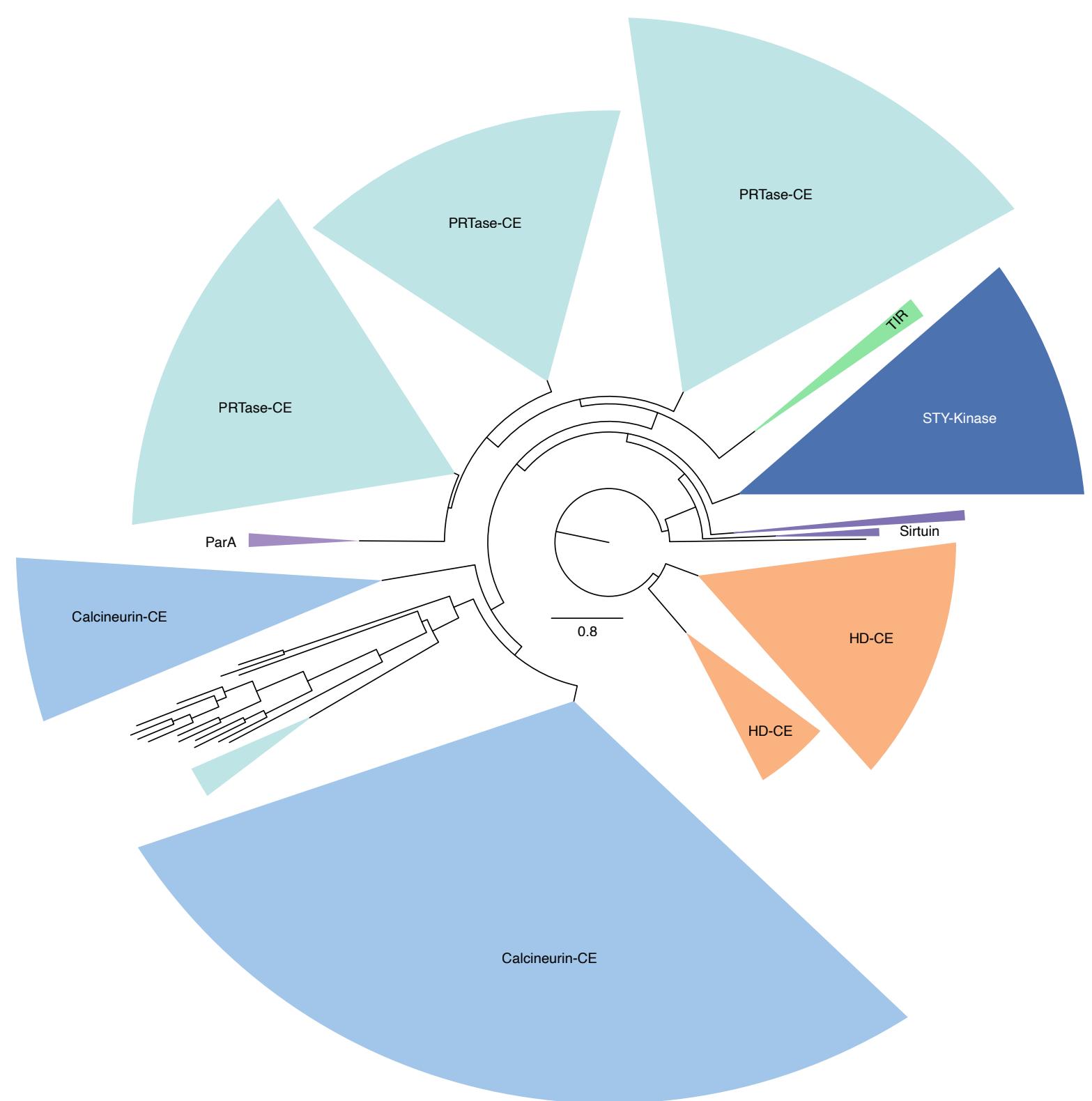
- 1. Calcineurin-CE
- 2. HD-CE
- 3. PRTase-CE
- 4. ORC-Cdc6



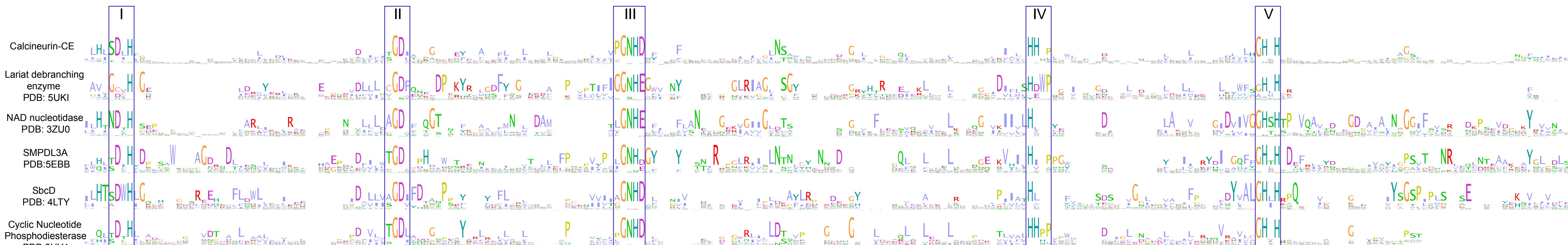
Supplementary Figure S1. Permuted GreAB-C structure model. (A) Structure of the previously identified GreAB-C domain. (B) Alphafold model of a permuted GreAB-C domain. (C) Context of the permuted GreAB-C domain embedded in a sequence of divergent TPRs, forming a toroidal structure.



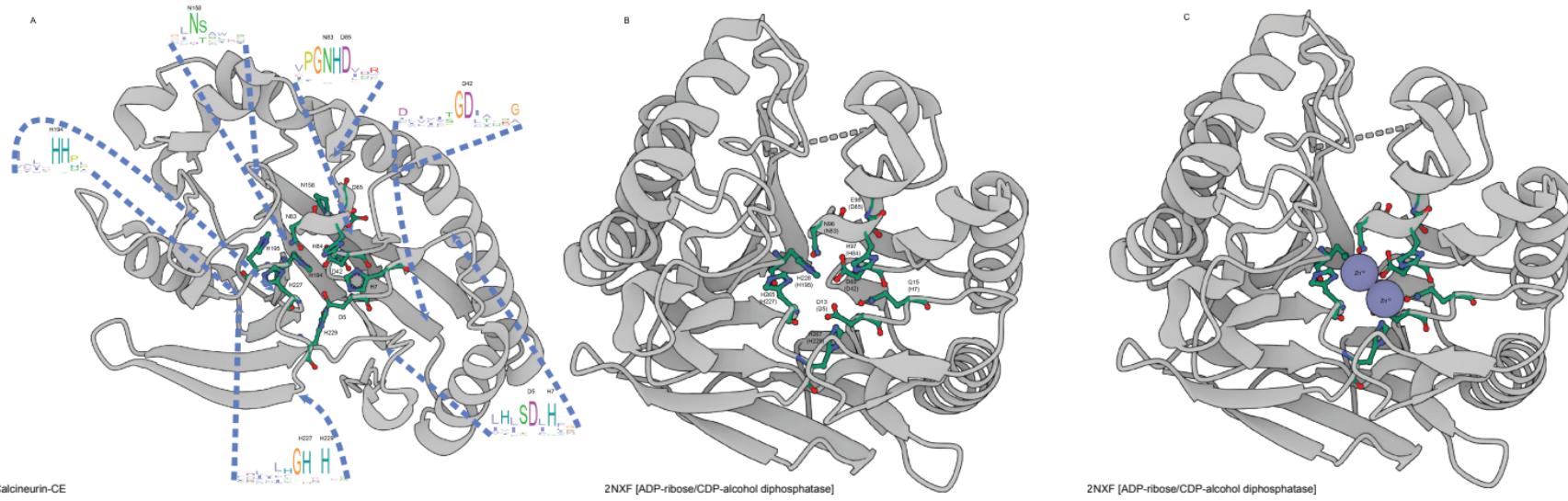
Supplementary Figure S2. Double PIN structure model. (A) cartoon representation of the double PIN domain. β -strands are depicted in yellow, α -helices in dark pink, and the HTH domain in dark cyan. Catalytic residues are shown in ball-and-stick representation. (B) Molecular surface representation demonstrating the occlusion of the active PIN site by the HTH domain. The two PIN domains are represented in purple, the HTH domain in green, and the position of the inactive catalytic residues of the inactive PIN domain is depicted in red.



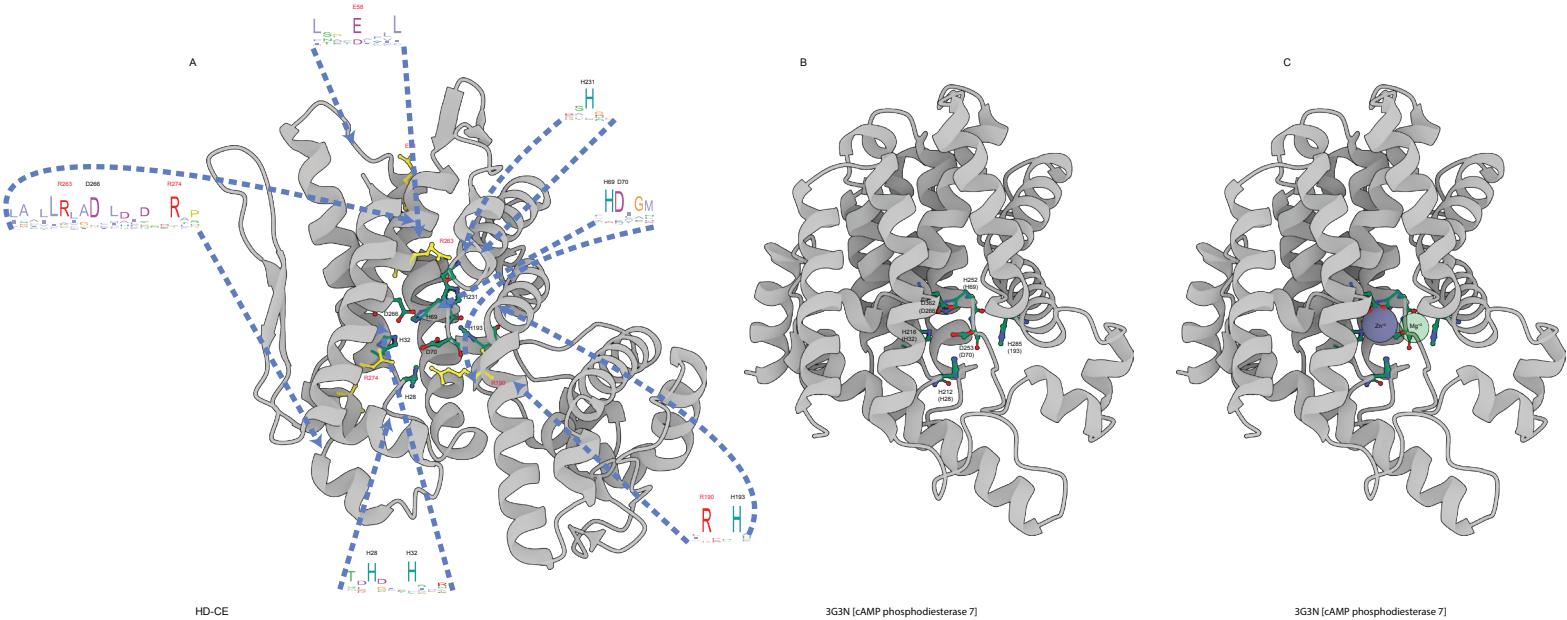
Supplementary Figure S3. Phylogenetic tree of the Orc/Cdc6-like proteins. The tree was constructed with IQtree2 using ultrafast bootstrap and SH-aLRT test as support values. The clades are labeled according to associated effector domains present in the genomic neighborhood



Supplementary Figure S4. Sequence logos of different Calcineurin-like phosphoesterase families. Sequences from various families of calcineurin-like domains were gathered using previously characterized PDB sequences as seeds. These sequences were aligned using the Mafft local pair algorithm, and the logos representing different families were visualized using the Jalview software. Conserved motifs I-V are depicted with boxes.

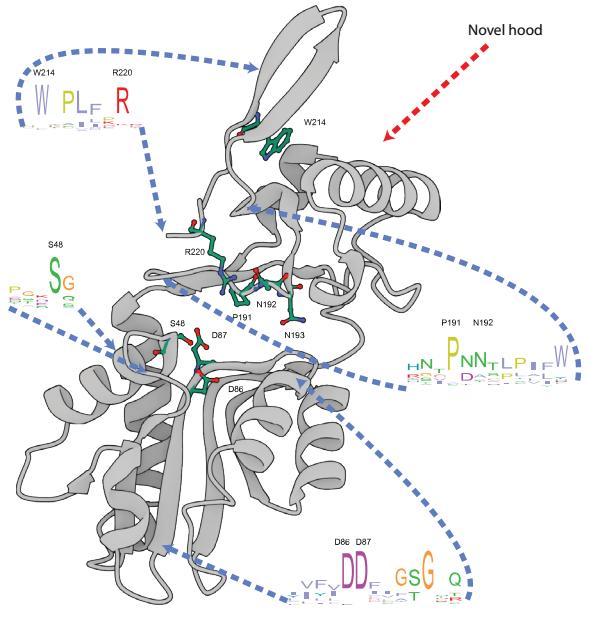


Supplementary Figure S5. Activity site and conserved residues from the Calcineurin-CE domain. (A) Alphafold modeling of the Calcineurin-CE domain (WP_080633877.1). The residues conserved in the activity sites are shown in ball and stick representations. The synapomorphic asparagine is colored in orange. Sequence logos of the conserved regions are shown and the dotted lines indicate the start and end position of the logo in the structural model. (B and C). Activity site of the experimental crystallized ADP-ribose/C-CDP-alcohol diphosphatase domain from the PDB 2NXF without and with the Zn⁺² ion respectively.



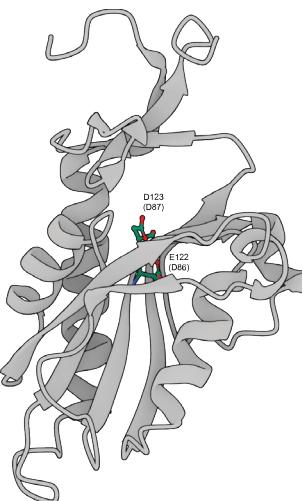
Supplementary Figure S6. Activity site and conserved residues from the HD-CE domain. (A) Alphafold modeling of the HD-CE domain (CZQ95560.1). The residues conserved in the activity sites are shown in ball and stick representations. The synapomorphic residues are colored yellow. Sequence logos of the conserved regions are shown and the dotted lines indicate the start and end position of the logo in the structural model. (B and C). Activity site of the experimental crystallized cAMP phosphodiesterase 7 domain from the PDB 3G3N without and with the Zn⁺² and MG⁺² ions respectively.

A



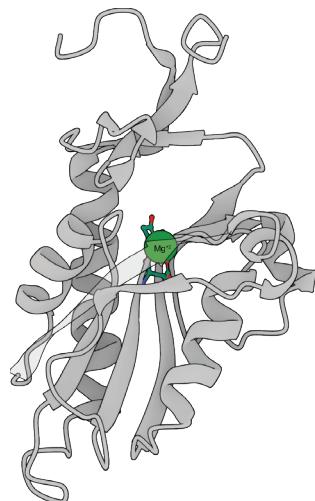
PRTase-CE

B



5KNQ [hypoxanthine guanine PRTase]

C



5KNQ [hypoxanthine guanine PRTase]

Supplementary Figure S7. Activity site and conserved residues from the PRTase-CE domain.
 (A) Alphafold modeling of the PRTase-CE domain (NPV02125.1). The residues conserved in the activity sites are shown in ball and stick representations. Sequence logos of the conserved regions are shown and the dotted lines indicate the start and end position of the logo in the structural model. (B and C). Activity site of the experimental crystallized hypoxanthine guanine PRTase domain from the PDB 5KNQ without and with the Mg⁺² ion respectively.

Figure 3A Calcineurin-CE

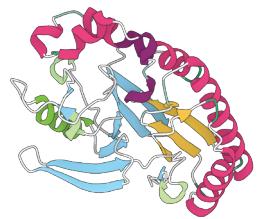
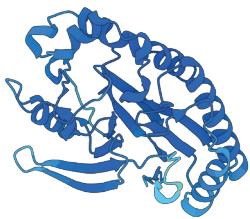


Figure 4G iSirtuin

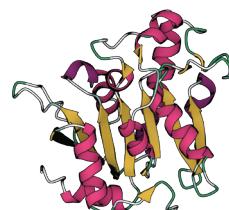
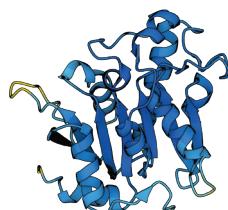


Figure 3B HD-CE

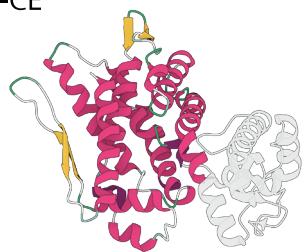
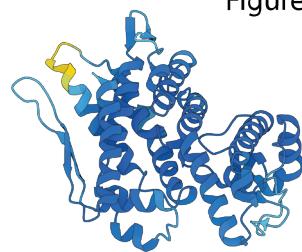


Figure 5G PRTase

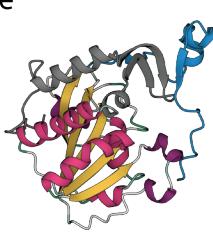
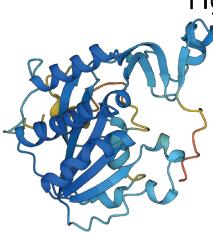


Figure 3C PRTase-CE



Figure 6B HSP90 system with C-terminal wHTH

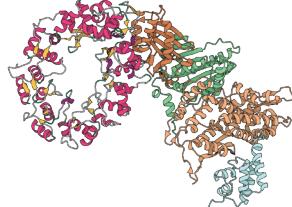
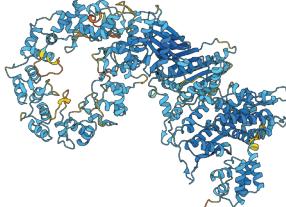


Figure 4B Cdc6•double-CARF•Calcineurin-CE complex

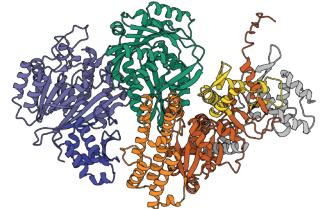
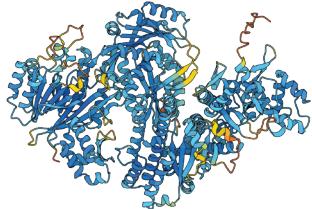


Figure 6E Pepco•Caspase complex



Figure 4D Pua-like

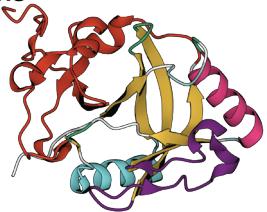
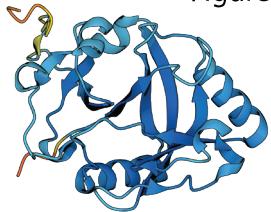
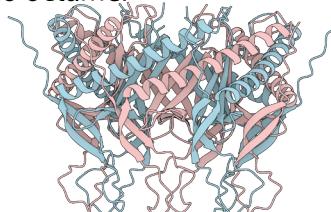
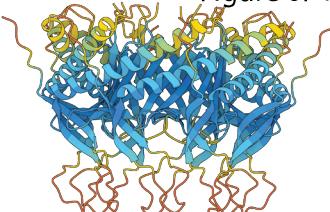


Figure 6F Pepco octamer



Very High (pLDDT > 90)

Confident (90 > pLDDT > 70)

Low (70 > pLDDT > 50)

Very Low(pLDDT < 50)

Supplementary Figure S8. AlphaFold confidence score for the models produced on the present work. On the left the structural models are colored by the pLDDT scores. On the right are the structural models as colored in the figures presented in the main text. Dark blue, light blue, yellow and orange represent a very high, confident, low and very low prediction respectively.

This block contains the full sequence of the 16S rRNA gene from the provided image, spanning from position 1 to approximately 980.

... (content of the file is extremely long and repetitive, containing thousands of entries for various species and their interactions)

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MBD9278			


Microbial community analysis									

... (content of the file is too large to be displayed here)

This block contains the full sequence of the 16S rRNA gene from the provided image, spanning from position 1 to approximately 980.


WP_169631302.1	ORC-CDC6-like	NZ_JABCLX010000258.1:1-326	GCF_012956145.1	108	*ORC-CDC6-like->	Vibrio parahaemolyticus	Gammaproteobacteria	NZ_JABC010000258
WP_162796445.1	ORC-CDC6-like	NZ_QEDE01000009.1:148204-153123	GCF_003347865.1	324	*ORC-CDC6-like->CARF-double->	Vibrio cholerae	Gammaproteobacteria	NZ_QEDE01000009.1
WP_165387721.1	ORC-CDC6-like	NZ_RBZD01000024.1:34261-41182	GCF_004214575.1	206	*ORC-CDC6-like->CARF-double->	Vibrio vulnificus	Gammaproteobacteria	NZ_RBZD01000024.1
WP_123300074.1	ORC-CDC6-like	NZ_RJVD01000001.1:67514-81212	GCF_003751465.1	655	PSE->*ORC-CDC6-like->	Vibrio crassostreiae	Gammaproteobacteria	NZ_RJVD01000001.1
RZP54190.1	ORC-CDC6-like	RZD01000024.1:34261-41081	GCA_004214575.1	173	*ORC-CDC6-like->CARF-double->	Vibrio vulnificus	Gammaproteobacteria	RZD01000024.1
TQP39892.1	ORC-CDC6-like	VIPA01000296.1:53-498	GCA_006802465.1	147	*ORC-CDC6-like->	Vibrio cholerae	Gammaproteobacteria	VIPA01000296.1
ALA88577.1	ORC-CDC6-like	CP011305.1:1884537-1900596	GCA_001274655.1	604	Na+TrkA_N->THF_DHG_CYH+THF_DHG_CYH_C->IMPDH->?->GATase+tRNA_Me_trans+GMP_synt_C->*ORC-CDC6-like->	Stenotrophomonas maltophilia	Gammaproteobacteria	CP011305.1
CAC9623431.1	ORC-CDC6-like	CAIZN01000037.1:54018-94433	GCA_903814045.1	559	PSE->?->SbcC->PSE->PSE->*ORC-CDC6-like->PSE->	uncultured Gammaproteobacteria bacterium	Gammaproteobacteria	CAIZN01000037.1
WP_078485038.1	ORC-CDC6-like	NZ_MPRL01000095.1:5394-12972	GCF_002020875.1	85	S-methyl_trans+Pterin_bind+B12-binding_2+B12-binding+Met_synt_B12->Glycos_transf_2->wHTH->*ORC-CDC6-like->	Solemya pervernica gill symbiont	Gammaproteobacteria	NZ_MPRL01000095.1
MBV8225564.1	ORC-CDC6-like	JAFAIU010000527.1:221-3464	GCA_019236535.1	320	*ORC-CDC6-like->CARF-double->	Verrucomicrobia bacterium	Verrucomicrobia	JAFAIU010000527.1
MBL9159074.1	ORC-CDC6-like	JAEUHI01000058.1:89930-100252	GCA_016795385.1	611	*ORC-CDC6-like->CARF-double->	Verrucomicrobiales bacterium	Verrucomicrobiae	JAEUHI01000058.1
MBL7075605.1	ORC-CDC6-like	JADHWK01000064.1:2665-12761	GCA_016783505.1	663	Phage_integrase->?->REC->*ORC-CDC6-like->?->CARF-double->tRNA->ncRNA->DNA_pol3_delta2+DNA_pol3_gamma3->YbaB_DNA_bd->RecR+Toprim_4->	candidate division KSB1 bacterium	candidate division KSB1	JADHWK01000064.1

NTSANDOS						
	nuclotide/dsD position	assembly	plen	organism	taxon	
					NCTC	
M0788707.1	Calineurin-CeSTANDS	M0788707.1	1,173	Actinobacteria bacterium	JADKCE010000010.1	
M0146G102.1	HO-CeSTANDS+P8_12	JADGCE010000010.1,14605...-32048	GCA_012717511.1	Magnococcace bacterium	JADGCE010000010.1	
A267775.1	Calineurin-CeSTANDS	K934860.1	1,602,829...-G1992	GCA_001851491.1	Methylotylococcus rosea	CPI04896.1
M07887083.1	Calineurin-CeSTANDS	M07887083.1	1,173,000...-1,173,373	GCA_012717511.1	Alphaproteobacteria	JADKCE010000010.1
M084779394.1	Calineurin-CeSTANDS	N_AVK010000011..1,248049..-302265	GCF_000887165.1	Skermonella aeroclast KACC 111604	JADPATE0000000000.1	
M014615137.1	Calineurin-CeSTANDS	N_AVK010000011..1,379524..-302265	GCF_000887165.1	Acidovorax oryzae ATCC 10882	JADPATE0000000000.1	
M07887084.1	Calineurin-CeSTANDS	M07887084.1	1,173,000...-1,173,373	GCA_012717511.1	Alcaligenes eutrophus bacterium	JADKCE010000010.1
M082535105.1	Calineurin-CeSTANDS	M082535105.1	1,209,000...-1,209,373	GCF_000887165.1	Acinetobacter lwoffii	JADPATE0000000000.1
M014640449.1	Calineurin-CeSTANDS	M014640449.1	1,173,000...-1,173,373	GCA_012717511.1	Pectinatus sphaericus	JADKCE010000010.1
M07887085.1	Calineurin-CeSTANDS	M07887085.1	1,173,000...-1,173,373	GCA_012717511.1	Streptomyces arboricola	JADKCE010000010.1
M07887086.1	Calineurin-CeSTANDS	M07887086.1	1,173,000...-1,173,373	GCF_000887165.1	Planteomyces bacterium	JADPATE0000000000.1
M07887087.1	Calineurin-CeSTANDS	M07887087.1	1,173,000...-1,173,373	GCA_012717511.1	Streptomyces olivochromogenes	JADKCE010000010.1
M07887088.1	Calineurin-CeSTANDS	M07887088.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887089.1	Calineurin-CeSTANDS	M07887089.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887090.1	Calineurin-CeSTANDS	M07887090.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887091.1	Calineurin-CeSTANDS	M07887091.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887092.1	Calineurin-CeSTANDS	M07887092.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887093.1	Calineurin-CeSTANDS	M07887093.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887094.1	Calineurin-CeSTANDS	M07887094.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887095.1	Calineurin-CeSTANDS	M07887095.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887096.1	Calineurin-CeSTANDS	M07887096.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887097.1	Calineurin-CeSTANDS	M07887097.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887098.1	Calineurin-CeSTANDS	M07887098.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887099.1	Calineurin-CeSTANDS	M07887099.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887100.1	Calineurin-CeSTANDS	M07887100.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887101.1	Calineurin-CeSTANDS	M07887101.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887102.1	Calineurin-CeSTANDS	M07887102.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887103.1	Calineurin-CeSTANDS	M07887103.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887104.1	Calineurin-CeSTANDS	M07887104.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887105.1	Calineurin-CeSTANDS	M07887105.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887106.1	Calineurin-CeSTANDS	M07887106.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887107.1	Calineurin-CeSTANDS	M07887107.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887108.1	Calineurin-CeSTANDS	M07887108.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887109.1	Calineurin-CeSTANDS	M07887109.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887110.1	Calineurin-CeSTANDS	M07887110.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887111.1	Calineurin-CeSTANDS	M07887111.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887112.1	Calineurin-CeSTANDS	M07887112.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887113.1	Calineurin-CeSTANDS	M07887113.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887114.1	Calineurin-CeSTANDS	M07887114.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887115.1	Calineurin-CeSTANDS	M07887115.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887116.1	Calineurin-CeSTANDS	M07887116.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887117.1	Calineurin-CeSTANDS	M07887117.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887118.1	Calineurin-CeSTANDS	M07887118.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887119.1	Calineurin-CeSTANDS	M07887119.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887120.1	Calineurin-CeSTANDS	M07887120.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887121.1	Calineurin-CeSTANDS	M07887121.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887122.1	Calineurin-CeSTANDS	M07887122.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887123.1	Calineurin-CeSTANDS	M07887123.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887124.1	Calineurin-CeSTANDS	M07887124.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887125.1	Calineurin-CeSTANDS	M07887125.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887126.1	Calineurin-CeSTANDS	M07887126.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887127.1	Calineurin-CeSTANDS	M07887127.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887128.1	Calineurin-CeSTANDS	M07887128.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887129.1	Calineurin-CeSTANDS	M07887129.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887130.1	Calineurin-CeSTANDS	M07887130.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887131.1	Calineurin-CeSTANDS	M07887131.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887132.1	Calineurin-CeSTANDS	M07887132.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887133.1	Calineurin-CeSTANDS	M07887133.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887134.1	Calineurin-CeSTANDS	M07887134.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887135.1	Calineurin-CeSTANDS	M07887135.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887136.1	Calineurin-CeSTANDS	M07887136.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE0000000000.1
M07887137.1	Calineurin-CeSTANDS	M07887137.1	1,173,000...-1,173,373	GCF_000887165.1	Streptomyces sp.	JADPATE000









1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

