Metagenome mining and functional analysis reveal oxidized guanine DNA repair at the Lost City Hydrothermal Field

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Abstract

The GO DNA repair system protects against GC → TA mutations by finding and removing oxidized guanine. The system is mechanistically well understood but its origins are unknown. We searched metagenomes and abundantly found the genes encoding GO DNA repair at the Lost City Hydrothermal Field (LCHF). We recombinantly expressed the final enzyme in the system to show MutY homologs function to suppress mutations. Microbes at the LCHF thrive without sunlight, fueled by the products of geochemical transformations of seafloor rocks, under conditions believed to resemble a young Earth. High levels of the reductant H_2 and low levels of O_2 in this environment raise the question, why are resident microbes equipped to repair damage caused by oxidative stress? MutY genes could be assigned to metagenome assembled genomes (MAGs), and thereby associate GO DNA repair with metabolic pathways that generate reactive oxygen, nitrogen and sulfur species. Our results indicate that cell-based life was under evolutionary pressure to cope with oxidized guanine well before O_2 levels rose following the great oxidation event.

Introduction

The Lost City Hydrothermal Field (LCHF) resembles conditions of a younger planet and thus provides a window to study the origin of life on Earth and other planets (1–3). Located 15 kilometers from the Mid-Atlantic Ridge, the LCHF comprises a series of carbonate chimneys at depths ranging from 700-800 meters below the ocean surface (3). The temperature and pH of the LCHF are both elevated, with temperature ranging from 40°C to 90°C and pH ranging from 9 to 11 (1). At this depth, light does not penetrate, magmatic energy sources are unavailable, and

41 dissolved carbon dioxide is scarce (1). Despite these environmental constraints, archaea and
42 bacteria inhabit the chimneys and hydrothermal fluids venting from the subsurface (1,4). The
43 chemoautotrophic microbes take advantage of chemical compounds generated by subseafloor
44 geochemical reactions such as serpentinization, the aqueous alteration of ultramafic rocks (1).
45 Serpentinization produces hydrogen gas and low-molecular-weight hydrocarbons, which fuel
46 modern microbial communities and also would have been needed to fuel self-replicating
47 molecules and the emergence of primitive metabolic pathways as an antecedent to cellular life
48 (1,2,5). Hydrothermal circulation underneath the LCHF depletes seawater oxygen, leading to an
49 anoxic hydrothermal environment very different from the nearby oxygen-rich seawater (1,3,6–8).
50 As such, the subsurface microbial communities may offer a glimpse into how life emerged and
51 existed before the Great Oxidation Event that occurred over two billion years ago.
52 The unusual environmental conditions of the LCHF present several biochemical
53 challenges to the survival of microbes (4,7,8). For example, high temperatures and alkaline pH
54 conditions present at the LCHF potentiate chemistry to generate DNA-damaging, reactive
55 oxygen species (9). However, it remains unclear whether reactive oxygen species (ROS) are a
56 major threat to resident microbes given that the subseafloor underneath the LCHF is largely
57 devoid of molecular oxygen. We reasoned that the prevalence or absence of DNA repair
58 pathways that cope with oxidative damage would provide insight to the question, are ROS a real
59 and present danger to life at the LCHF?
60 The guanine oxidation (GO) DNA repair system addresses the most common type of
61 DNA damage caused by reactive oxygen species, the 8-oxo-7,8-dihydroguanine (OG)
62 promutagen (**Fig 1**) (10). The OG base differs from guanine by addition of only two atoms, but
63 these change the hydrogen bonding properties so that OG pairs equally well with cytosine and

64 adenine during DNA replication. The resulting OG:A lesions fuel G:C → T:A transversion

65 mutations if not intercepted by the GO DNA repair system (9). The GO system comprises

66 enzymes encoded by *mutT*, *mutM*, and *mutY*, first discovered through genetic analyses of

67 *Escherichia coli* that demonstrated specific protection from G:C → T:A mutations by these three

68 genes (11–14). Homologs or functional equivalents of these GO system components are found

69 throughout all three kingdoms of life (15–17), underscoring the importance of the system, yet

70 there are several instances where particular bacteria (18,19) or eukaryotes (20) make do without

71 one or more of these genes.

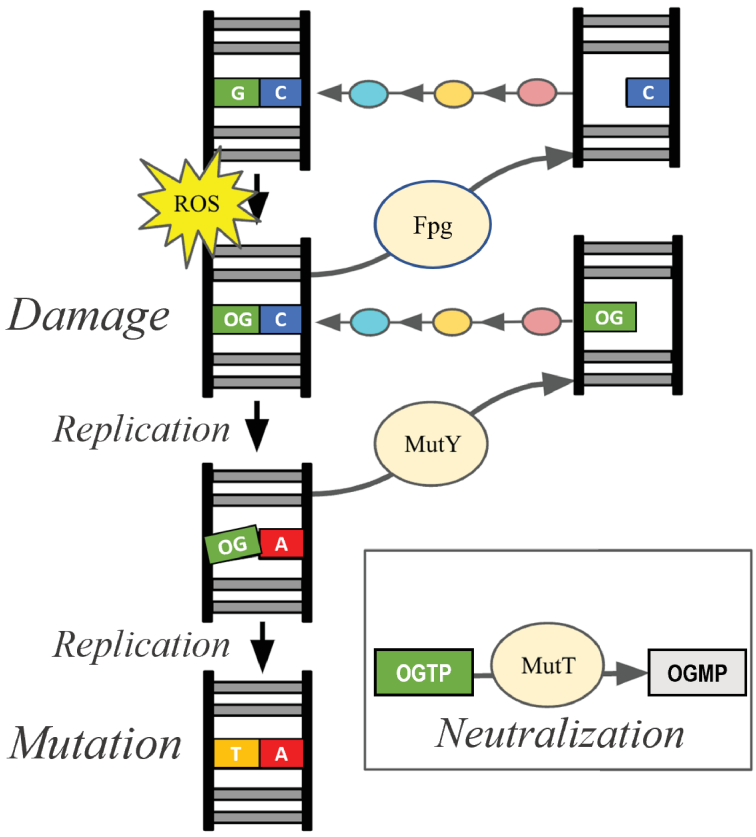

Fig 1. Overview of the GO system. The gene products of *mutT*, *mutM*, and *mutY* (tan bubbles) prevent or repair oxidized guanine DNA damage caused by ROS. DNA glycosylases Fpg (encoded by *mutM*) and MutY remove OG from OG:C and A from OG:A, respectively, to create AP sites with no base. Additional enzymes (AP nucleases, pink; DNA polymerase, orange; and DNA ligase, teal) cooperate with the GO pathway, process these AP sites and ultimately restore the GC base pair. MutT neutralizes OG nucleotide triphosphates to prevent incorporation of the OG nucleotide during DNA replication, thereby ensuring that OG found in DNA is on the parent strand, not the daughter strand.

72

73 Biochemical analyses of gene products have provided a complete mechanistic picture for

74 the GO repair system. MutT hydrolyzes the OG nucleotide triphosphate to sanitize the nucleotide

75 pool, thus limiting incorporation of the promutagen into DNA by DNA polymerase (13,21). The

76 enzyme encoded by *mutM*, called formamidopyrimidine-DNA glycosylase (Fpg), locates OG:C
77 base pairs and excises the OG base to initiate base excision repair (BER) (12,22). MutY locates
78 OG:A lesions and excises the A base to initiate BER (11,14). Fpg and MutY thus act separately
79 on two different intermediates to prevent G:C → T:A mutations. These DNA glycosylases
80 generate abasic (apurinic/apyrimidinic; AP) sites, which are themselves mutagenic if not
81 processed by downstream, general BER enzymes, particularly AP nucleases (e.g. exonuclease III
82 and endonuclease IV), DNA polymerase and DNA ligase (17,23–25) as shown in **Fig 1**.
83 MutY is the final safeguard of the GO system. If left uncorrected, replication of OG:A
84 lesions results in permanent G:C → T:A transversion mutations as demonstrated by *mutY* loss of
85 function mutants (26,27). Underperformance of the mammalian homolog, MUTYH, leads to
86 early onset cancer in humans, first discovered for a class of colon cancers now recognized as
87 MUTYH Associated Polyposis (28). MutY is made up of two domains that both contribute to
88 DNA binding and biochemical functions. The N-terminal catalytic domain shares structural
89 homology with EndoIII and other members of the Helix-hairpin-Helix (HhH) protein
90 superfamily (17). The C-terminal OG-recognition domain shares structural homology with MutT
91 and other NUDIX hydrolase family members (17,29). Functionally important and highly
92 conserved residues define chemical motifs in both domains (**Fig 2**). These chemical motifs
93 interact with the OG:A lesion and chelate the iron-sulfur cluster cofactor as revealed by x-ray
94 structural analysis (**Fig 2**) (30–33). For example, residues in the N-terminal domain establish the
95 catalytic mechanism for adenine excision (**Fig 2A** and **Fig 2B**) (32,34). Residues found in a beta
96 loop of the C-terminal domain recognize the OG base and thus direct adenine removal from
97 OG:A lesions (**Fig 2C**) (33). Some motifs are shared among other DNA glycosylases, such as the
98 residues that chelate the 4Fe4S iron-sulfur cluster cofactor (**Fig 2D**) (17). Chemical motifs

99 particular to MutY, especially the OG-recognition residue Ser308 (**Fig 2C**) and supporting

100 residues in the C-terminal domain, are conserved across organisms and are not found in other

101 DNA glycosylases and therefore can be used to identify MutY genes (17).

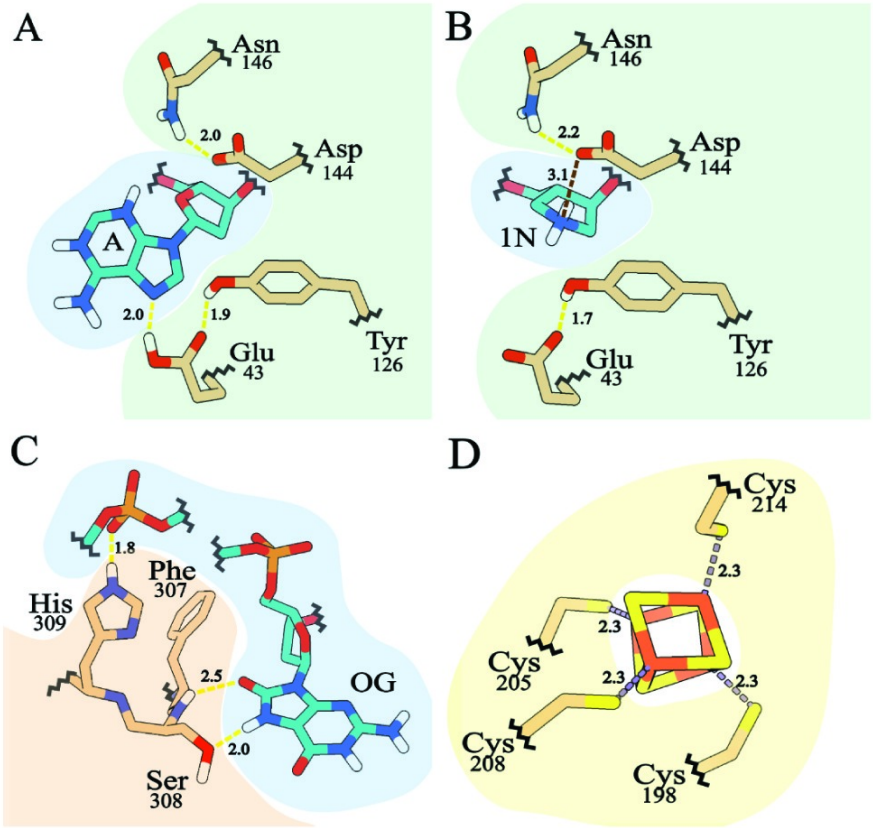

Fig 2. MutY chemical motifs. Panels A-C show interactions between MutY residues and DNA, with DNA highlighted in blue. Panel A was made from PDB ID 3g0q, a structure which describes interaction with the competitive inhibitor fluoro-A (31); panels B-D were made from PDB ID 6u7t, a structure which describes interaction with a transition state analog (1N) of the oxacarbenium ion formed during catalysis of adenine base excision (32). MutY catalytic residues (highlighted in green) interact with an adenine base (panel A) and the 1N moiety which mimics the charge and shape of the transition state (panel B). The OG-recognition residues (highlighted in orange) provide hydrogen bonding interactions with the OG base (panel C). Four cysteine residues chelate the iron-sulfur metal cofactor (panel D). All of these interactions are important for MutY activity.

102

103 Our study investigated whether microbes in the anoxic LCHF environment use the GO

104 DNA repair system to mitigate damage caused by reactive oxygen species. It is important to note

105 that not all organisms have an intact GO repair system; examples are missing one, some or all

106 components. MutY in particular was absent frequently in a survey of 699 bacterial genomes (19),
107 and its absence may indicate relaxed evolutionary selection from oxidized guanine damage (18).
108 We mined for homologous genes within the LCHF microbial community and recombinantly
109 expressed candidate MutY enzymes to characterize function. We found genes encoding GO
110 system components and general base excision repair enzymes at all LCHF sites. MutY homologs
111 from the LCHF suppressed mutations when expressed in *mutY* deficient *E. coli* strains, indicating
112 these function similarly to authentic MutY. These Lost City MutY homologs could be assigned
113 confidently to metagenome assembled genomes (MAG)s, allowing for additional gene inventory
114 analyses that revealed metabolic strategies involving sulfur oxidation and nitrogen reduction.
115 These results have important implications for understanding the repair of oxidative guanine
116 damage in low-oxygen environments, similar to those that existed on a younger Earth, as well as
117 those that may exist on other planets and moons.
118

119 Results

120 Identification of the GO DNA repair system in LCHF microbes

121 To investigate the potential for LCHF microbes to endure DNA damage caused by ROS
122 despite inhabiting a low oxygen environment, we searched for the GO DNA repair system in
123 metagenomes obtained from LCHF hydrothermal fluids (35). We identified gene homologs for
124 *mutT*, *mutM*, and *mutY*, which constitute the complete GO system (**Fig 3)**. The relative
125 abundances of these GO system gene homologs were similar to that of two other DNA repair
126 enzymes that were also frequently found in LCHF metagenomes. Exonuclease III and
127 endonuclease IV work in conjunction with the GO system and perform general functions

128 necessary for all base excision repair pathways, namely the processing of AP sites (24). MutY

129 was underrepresented in each of two samples from a chimney named "Marker 3", indicating that

130 this GO system component is not encoded by some of the LCHF residents (**Fig 3**, pink).

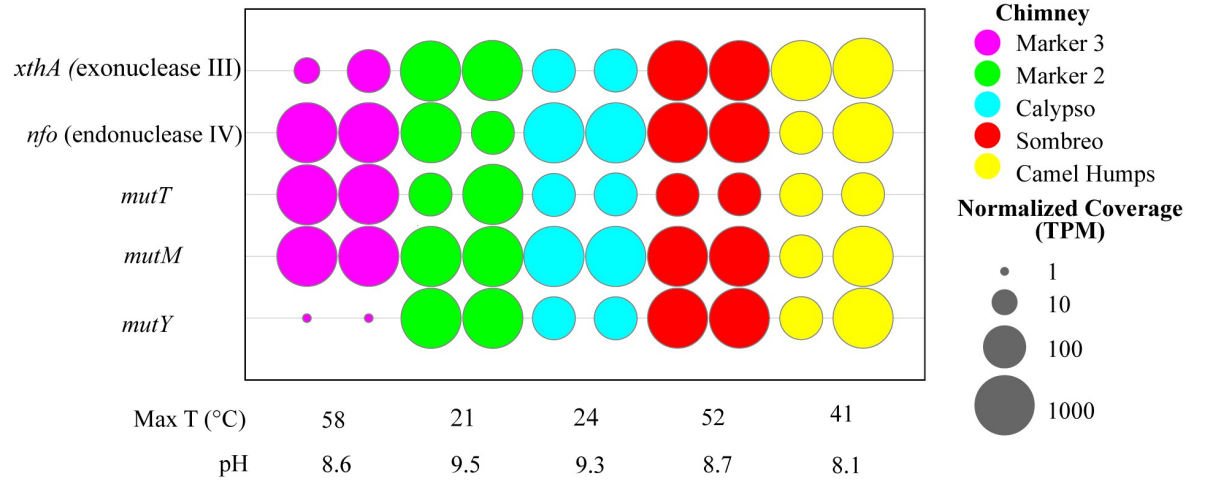

Fig 3. Abundance of GO system gene homologs. Listed on the vertical axis are genes encoding DNA repair enzymes. Genes *xthA* and *nfo* are generally necessary for DNA repair involving base excision repair in bacteria, including the particular GO system investigated here. Together, *mutT*, *mutM* and *mutY* constitute the GO system that deals specifically with oxidized guanine. Across the horizontal axis are the various LCHF sites, coded by color, from which samples were collected in duplicate, along with the reported temperature and pH. The normalized coverage of each gene is reported as a proportional unit suitable for cross-sample comparisons, the transcripts/fragments per million (TPM).

131

132 Metagenomic mining for MutY genes

133 Having determined that GO system gene homologs are abundant at the LCHF, we

134 focused our efforts on the final safeguard of the pathway, MutY. A BLASTP search against the

135 LCHF metagenome with query MutY sequences from *Geobacillus stearothermophilus (Gs*

136 MutY) and *E. coli* (*Ec* MutY) preliminarily identified 649 putative MutY candidates on the basis

137 of sequence identity, excluding hits with less than a 30% sequence identity cut-off or E-values

138 exceeding 1E-5 (**Fig 4A**). Structure-guided alignments of these preliminary hits were examined

139 for presence and absence of MutY-defining chemical motifs. We paid particular attention to the

140 chemical motif associated with OG recognition as these residues in the C-terminal domain

141 establish OG:A specificity, which is the hallmark of MutY (29,33,36). This approach

142 authenticated 160 LCHF MutYs (**Fig 4B**). Four representative LCHF MutYs were selected for

143 further analyses described below (red branches in **Fig 4A** and **Fig 4B**).

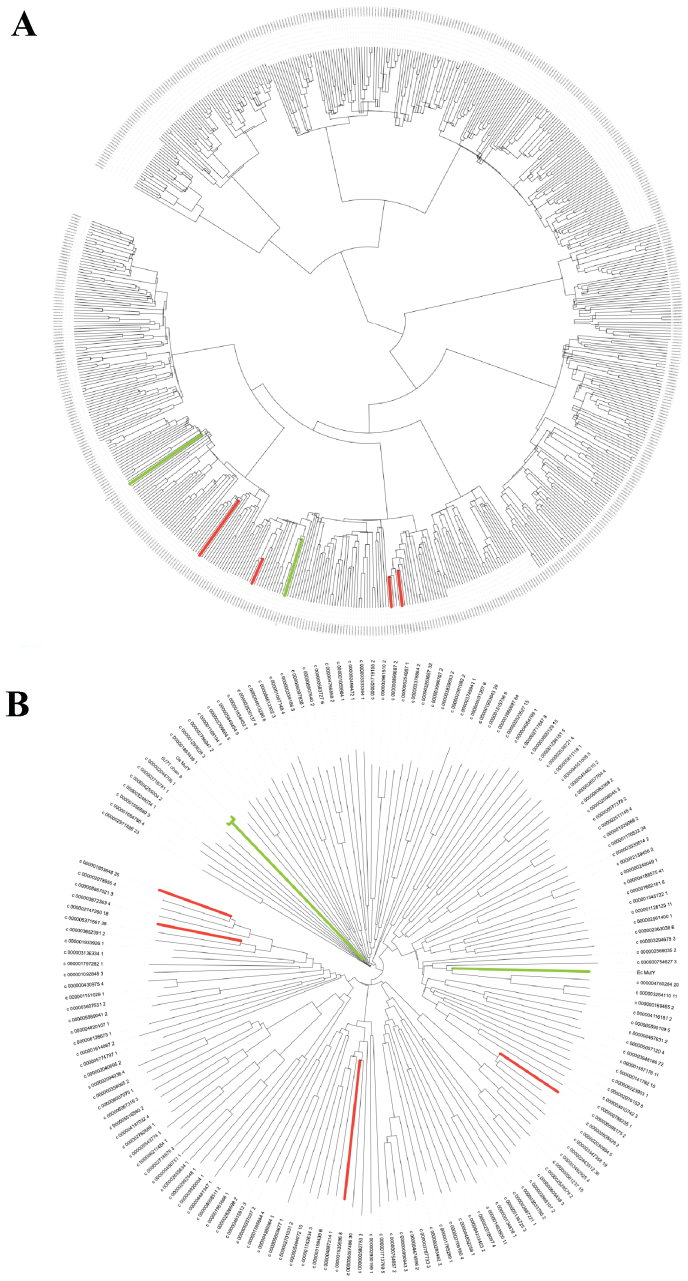

Fig 4. Phylogeny for LCHF MutYs. (A) 649 sequences were identified as LCHF MutY candidates due to sequence similarity to existing MutYs (green branches) and aligned to reconstruct evolutionary relationships. (B) A subset of 160 members contained all necessary MutY-defining chemical motifs. Alignment of these authenticated LCHF MutYs revealed varying evolutionary distances from familiar MutYs and provided a basis for selecting four representative members (red branches).

144

145 **Fig 5** highlights conservation and diversity for the MutY-defining chemical motifs found

146 in the 160 LCHF MutYs. All of the LCHF MutYs retain the chemical motif to coordinate the

147 iron-sulfur cluster cofactor comprising 4 invariant Cys residues (4 Cs, yellow in **Fig 5**), a feature

148 that is also found in other HhH family members such as EndoIII (16), but which is absent for

149 some "clusterless" MutYs (37). Other invariant and highly conserved motifs make critical

150 interaction with the DNA and provide key catalytic functions for adenine base excision,

151 explaining the high degree of sequence conservation at these positions.

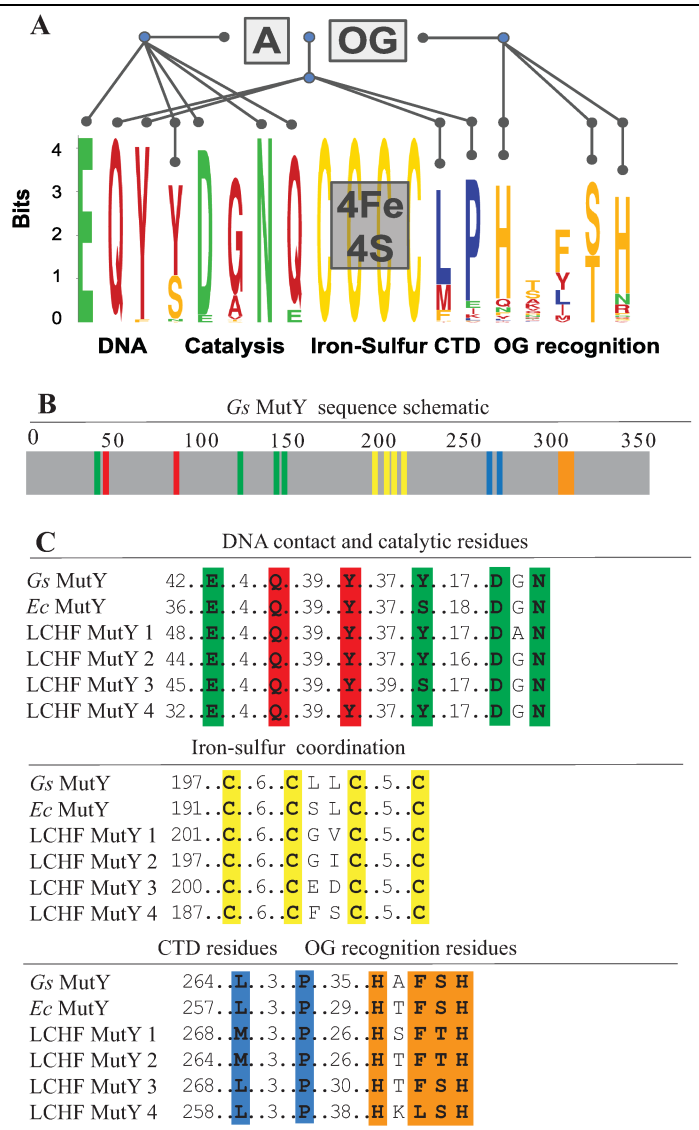

Fig 5. LCHF MutY chemical motifs. (A) Conservation and diversity of MutY-defining chemical motifs are depicted with a sequence logo for the 160 LCHF MutYs. These motifs are associated with biochemical functions including DNA binding, enzyme catalysis, attachment of the iron-sulfur cofactor, and recognition of the damaged OG base. Sequence logo generated by Weblogo (38,39). See **Supporting Information** for a complete alignment (**Dataset S1**) and a percent identity matrix for the representative LCHF MutYs (**Table S2**). (B) Chemical motifs located in MutY as shown with color-coded positions for *Gs* MutY. (C) Alignment for select chemical motifs highlights conservation among the four representative LCHF MutYs, *Ec* MutY and *Gs* MutY.

152 For example, all LCHF MutYs use a Glu residue which provides acid base catalysis for
153 the mechanism (first E, green in **Fig 5**). Also, all 160 LCHF MutYs use a Gln (first Q, red in **Fig**
154 **5**) and all but one use a Tyr (Y/F, red/gold in **Fig 5A**), to wedge between base pairs and thereby
155 distort the DNA for access to the adenine as seen in x-ray crystal structures of *Gs* MutY (30,31).
156 Structures of *Gs* MutY interacting with a transition state analog revealed close contact with
157 Tyr126, which is often Ser as in *Ec* MutY, Asp144, and supported by Asn146, indicating these
158 chemical motifs stabilize the transition state during catalysis (32–34). For the LCHF MutYs, the
159 Asn residue is invariant (N, green in **Fig 5**), the catalytic Asp is nearly invariant (D/E, green in
160 **Fig 5A**), replaced by chemically similar Glu for seven LCHF MutYs, and the catalytic Tyr
161 residue is often replaced by Ser and sometimes by Thr or Asn (Y/S/N, red/gold/green in **Fig 5A**).
162 The residue found between the catalytic Asp and Asn is always a small residue, most often Gly
163 but sometimes Ala, Val, Thr or Ser (G/A/V, red in **Fig 5A**) .
164 By contrast with these numerous and highly conserved motifs for the N-terminal domain,
165 fewer motifs with greater sequence divergence were found in the C-terminal domain. The MutY
166 ancestor is thought to have resulted from a gene fusion event that attached a MutT-like domain to
167 the C-terminus of a general adenine glycosylase enzyme to confer OG:A specificity as observed
168 for modern *Ec* MutY (29). X-ray crystal structures of *Gs* MutY interacting with OG:A and with
169 G:A highlighted conformational difference for a Ser residue in the C-terminal domain (Ser308 in
170 *Gs* MutY), and mutational analysis showed this Ser residue and its close neighbors (Phe307 and
171 His309 in *Gs* MutY) establish OG specificity (33). Informed by these insights from structure, we
172 eliminated LCHF MutY candidates that lacked a C-terminal domain and its OG-recognition
173 motif. Alignment of the 160 LCHF MutYs that passed this test showed that a second His residue
174 is also well conserved within the H-x-FSH sequence motif (H/Q/N, gold/red/green in **Fig 5A**).

175 As is evident from the alignment, there are many variations with FSH residues replaced by close
176 analogs at each position. Ser, which makes the key contacts with N7 and O8 of OG and no
177 contact with undamaged G, is often replaced by Thr (S/T, gold in **Fig 5A**). Likewise the His
178 residue is often replaced by polar residues such as Asn, Arg or Lys (H/N/R, gold/green/red in**Fig**
179 **5A**) that can also hydrogen bond to the DNA phosphate backbone as observed for His309 in *Gs*
180 MutY.

181 Two other positions with high conservation were revealed for the C-terminal domain in
182 this analysis of the 160 LCHF MutYs. These define a L-xxx-P motif. These residues are replaced
183 by other residues with comparable chemical properties. The Leu position is often another
184 hydrophobic residue such as Met or Phe (L/M/F, blue/red/gold in **Fig 5A**), and the Pro position is
185 most frequently replaced by Glu (P/E, blue/green in **Fig 5A**), a residue that can present aliphatic
186 methylene groups and thus resemble Pro if the polar group hydrogen bonds with the peptide
187 amide. In the structure of *Gs* MutY, the Pro269 nucleates a hydrophobic core for the C-terminal
188 domain. The Leu265 makes a strong VDW contact with Tyr89 in the N-terminal domain to
189 support stacking of Tyr88 between bases of the DNA, a molecular contact that suggests
190 communication between the OG-recognition domain and the catalytic domain. Other
191 evolutionary analyses have highlighted the motifs important for DNA contacts, catalysis and OG
192 recognition (17), but to our knowledge the L-xxx-P motif has not been identified previously.

193 Four representative LCHF MutYs were selected for further analyses. Supporting
194 Information **Table S2** reports the percent identity among these representative LCHF MutYs and
195 the well-studied MutYs from *E. coli* and *Geobacillus stearothermophilus*. LCHF MutY 1 and
196 LCHF MutY2 are most closely related with 65% identity which is almost twice the average in
197 this group. LCHF MutY 3 is most closely related to *Ec* MutY with 48% identity. We examined

198 the representative LCHF MutYs for physical properties as inferred from sequences. **Table 1**
199 reports these physical properties including predicted protein size, isoelectric point (pI), and
200 stability (Tm). Generally the physical characteristics measured for LCHF MutY representatives
201 were comparable to each other and to predicted properties of *Ec* MutY and *Gs* MutY. The
202 predicted Tm for LCHF MutY 3 was above 65°C, distinguishing it as the most stable enzyme
203 (**Table 1**), which may reflect adaptation to a high temperature environment. The isoelectric point
204 predicted for each of the LCHF MutY representatives is 3 pH units above the pI predicted for *Gs*
205 MutY and between 0.1 - 0.5 pH units above the pI predicted for *Ec* MutY, indicating that more
206 numerous positively charged residues have been recruited, possibly as an adaptation to the LCHF
207 environment.
208
209 **Table 1. Physical protein properties**

MutY	Length (residues)	MW (kDa)	pI	Predicted Tm (°C) (NTD; CTD)
Gs MutY	372	41.8	5.3	55 - 65 (55 - 65 ; < 55)
Ec MutY	355	39.1	8.6	< 55 (< 55 ; 55 - 65)
LCHF MutY 1	358	39.0	9.1	55 - 65 (55 - 65 ; < 55)
LCHF MutY 2	352	38.3	8.8	< 55 (55 - 65 ; < 55)
LCHF MutY 3	370	42.0	8.7	> 65 (>65 ; 55 - 65)
LCHF MutY 4	376	44.0	9.0	55 – 65 (55 - 65 ; 55 - 65)

210

211 Identification of LCHF MutY organisms, gene neighbors, 212 environmental conditions, and metabolic strategies

213 Our next objective was to identify the organisms from which these LCHF MutY enzymes
214 originated. Each of the four representative LCHF MutY sequences were derived from contiguous

215 DNA sequences (contigs) belonging to a metagenome-assembled genome (MAG) representing a
216 LCHF microbe. The taxonomic classification of these MAGs indicated that LCHF MutY 1
217 originated from a species of *Marinosulfonomonas,* LCHF MutY 2 from the family
218 *Rhodobacteraceae*, LCHF MutY 3 from the family *Thiotrichaceae*, and LCHF MutY 4 from the
219 family *Flavobacteriaceae* (**Fig 6**). The taxonomic classification of each contig was consistent
220 with the classification of the MAG to which it belonged, supporting the idea that the MutY gene
221 is a long term resident and not a recent arrival through phage infection or some other horizontal
222 gene transfer mechanism. For the remainder of this work we will refer to the MutY-encoding
223 organisms by the lowest-level classification that was determined for each LCHF MutY (e.g.
224 LCHF MutY 3 will now be referred to as *Thiotrichaceae* MutY).

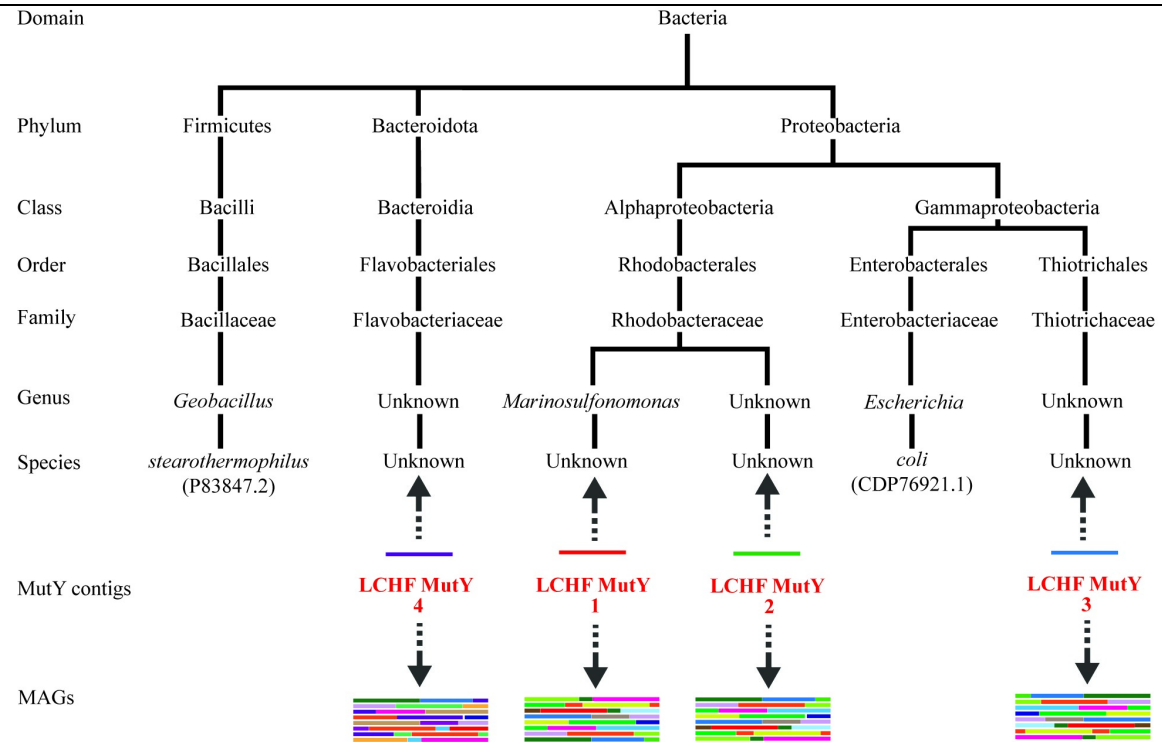

Fig 6. Taxonomic classification. LCHF MutY-encoding contigs were found in several branches of bacteria. The classification places these in relation to MutY of *G. stearothermophilus* and *E. coli* (accession IDs included). LCHF MutYs were mapped to their respective microbes by two methods indicated by the two arrows (see text for details).

225 The inclusion of MutY contigs in MAGs provided an opportunity to examine gene
226 neighbors for the representative LCHF MutYs. The GO repair genes are located at distant loci in
227 *E. coli* (12), and belong to separate operons (40). However, MutY is the immediate 5'-neighbor
228 to YggX within gammaproteobacteria (40), and homologs of YggX are present outside this
229 lineage, occasionally nearby to MutY (e.g. *Bacillus subtilis*). As gene neighbors, MutY and
230 YggX are part of a SoxRS regulated operon in *E. coli* (40,41). YggX provides oxidative stress
231 protection and iron transport function with a critical Cys residue close to the N-terminus of this
232 small protein (42,43). A protein matching these features is encoded by a gene partly overlapping
233 with and the nearest 3' neighbor to *Thiotrichaceae* MutY (see Supporting Information **Fig S3**).
234 To reveal the environmental conditions of these MutY-encoding organisms, we analyzed
235 the sequence coverage of each LCHF MutY contig at each of the sampling sites.
236 *Marinosulfonomonas* MutY, *Thiotrichaceae* MutY, and *Flavobacteriaceae* MutY were identified
237 at all sampling sites, ranging from 21°C to 58°C and pH 8.1 to 9.5. *Rhodobacteraceae* MutY was
238 present at all sampling sites excluding Marker 3 and was therefore found in temperatures ranging
239 from 24°C to 52°C and pH 8.1 to 9.5.
240 We further investigated the metabolic strategies utilized by MutY-encoding microbes by
241 examining the inventory of predicted protein functions in each MAG (**Table 2,** see also
242 Supporting Information **Table S4**). Each LCHF MutY-containing MAG possessed at least two
243 forms of cytochrome oxidase, with the exception of the *Flavobacteriaceae* MAG. The
244 *Flavobacteriaceae* MAG is only 44% complete, however, so no strong conclusions can be made
245 regarding the absence of genes. Cytochrome oxidases commonly provide sources of free radicals
246 and are essential to aerobic metabolism. Predicted proteins indicative of dissimilatory nitrate and
247 nitrite reduction were found in the *Marinosulfonomonas, Rhodobacteraceae,* and *Thiotrichaceae*

248 MAGs, suggesting that these organisms may be capable of using nitrate or nitrite as alternative
249 electron acceptors when oxygen is not available. Furthermore, the *Marinosulfonomonas* and
250 *Rhodobacteraceae* MAGs include predicted protein functions associated with the oxidation of
251 reduced sulfur compounds, though it is important to note that the directionality of these reactions
252 cannot be fully determined from bioinformatics alone. These patterns speak to the potential
253 origins of oxidants within the MutY-encoding organisms as discussed below.
254

255 **Table 2. Metabolic genes identified in LCHF MutY organisms**

			Marinosulfonomonas [a]		*Rhodobac-teraceae*	*Thiotrich-aceae*	*Flavobac-teriaceae*
			MAG 1	MAG 2			
Cytochrome Oxidases	UQCRFS1	K00411	X	X	X	X	
	coxA	K02274			X	X	
	ccoN	K00404	X	X	X		
	cydA	K00425		X			
	cyoB	K02298			X		
Sulfur Oxidation	soxA	K17222	X	X	X		
	soxX	K17223	X	X	X		
	soxB	K17224	X	X	X		
	soxC	K17225	X	X	X		
	soxY	K17226	X		X		
	soxZ	K17227	X		X		
Nitrogen Reduction	narG	K00370	X	X	X		
	narH	K00371	X	X	X		
	nirB	K00362		X	X	X	
	nirD	K00363		X		X	
	nirK	K00368			X		
	norB	K04561			X		
	norC	K02305			X		
	nosZ	K00376			X		
	MAG Completeness (%) [b]		88.4	88.2	93.7	66.1	44.3
	MAG Contamination (%) [b]		16.4	0.6	1.4	11.8	1.6

256
257 [a] *Marinosulfonomonas* MutY belongs to two separate MAGs and genes for each are reported separately.
258 [b] Completeness and contamination scores generated by *CheckM* v1.0.5 as described in Brazelton *et al.* 2022 (35).

Predicted protein structures and virtual docking experiments

To assess the likelihood of the LCHF MutY sequences folding into enzymes capable of activity on the OG:A substrate, protein structures were predicted using *Colabfold* (44)(**Fig 7**). These predicted structures were associated with high confidence as indicated by pLDDT scores and PAE profiles (**Table 3** and Supporting Information **Fig S5**). Superpositions revealed that the predicted structures for the LCHF MutYs are each highly comparable with the experimentally determined structure for *Gs* MutY as indicated by visual inspection (**Fig 7**) and by low, pairwise root mean square deviation (RMSD) values (**Table 3**). The whole protein superpositions were

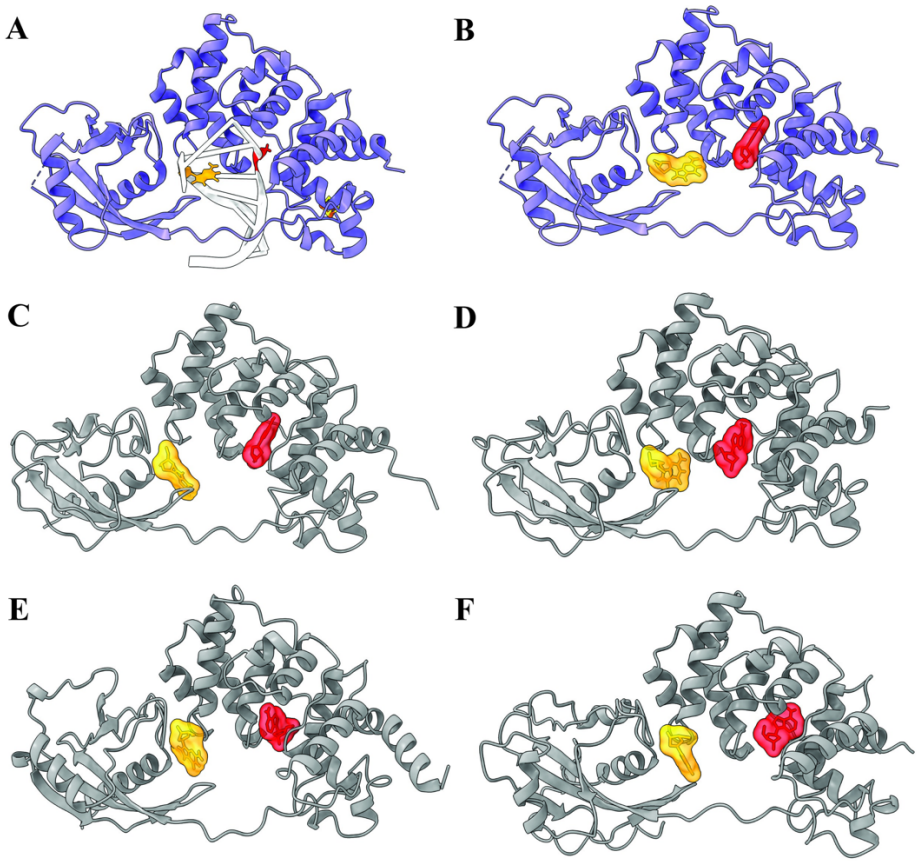

Fig 7. Structure predictions and virtual docking of MutY ligands. (A) The x-ray crystal structure of *Gs* MutY (blue ribbon; PDB ID 3g0q) in complex with DNA (white helix) highlights the positions of the adenosine (red) and OG (yellow). (B-F) Virtual docking of ligands. Adenosine and OG were separately docked to identify binding surfaces for these ligands in the structures of *Gs* MutY (B) which served as a positive control and the four representative LCHF MutYs: *Marinosulfonomonas* MutY (C); *Rhodobacteraceae* MutY (D); *Thiotrichaceae* MutY (E); and *Flavobacteriaceae* MutY (F).

268 dominated by the larger, more structurally conserved N-terminal domain. Breaking the analysis
269 into two separate domains showed that the C-terminal domain, although more plastic, retained
270 core structural features that could be superimposed. The MutY-defining chemical motifs are
271 positioned in locations similar to those seen for the *Gs* MutY reference structure, providing
272 evidence these LCHF MutY enzymes are capable of recognizing OG:A lesions and excising the
273 adenine base. Concisely, the LCHF MutY structure predictions resemble a functional MutY
274 enzyme from a thermophilic bacterium.
275
276 **Table 3. Molecular modeling for LCHF MutYs**

		RMSD (Å) [a] (residues)		Affinity VINA [b] (kcal/ mol)		Energy Amber [c] (kJ/mol)	
MutY Model	**pLDDT [d]**	**NTD**	**CTD**	**A**	**OG**	**A**	**OG**
Gs MutY (6u7t) [e]	NA	0.39 (221)	0.54 (117)	-7.3	-7.7	-193 (±33)	-181 (±26)
Marinosulfonomonas	93 (±9)	0.89 (202)	0.90 (64)	-6.8	-7.5	-130 (±36)	-122 (±66)
Rhodobacteraceae	94 (±6)	0.84 (209)	0.98 (51)	-6.8	-7.7	-156 (±24)	-178 (±41)
Thiotrichaceae	92 (±12)	1.0 (199)	1.1 (80)	-7.0	-8.0	-217 (±24)	-255 (±22)
Flavobacteriaceae	92 (±14)	0.92 (211)	0.88 (61)	-7.2	-8.0	-206 (±42)	-162 (±42)

277 [a] Root mean square deviation for the superposition of predicted structures with *Gs* MutY (PDB ID 3g0q) was
278 calculated separately for N-terminal domain (NTD) and C-terminal domain (CTD) by *ChimeraX* (45).
279
280 [b] Binding affinity for the best outcome from docking adenosine to the enzyme active site and OG to the OG-
281 recognition site as calculated by *Autodock VINA* (46,47).
282
283 [c] Energy for short-range Coulombic and Lennard-Jones interactions averaged over a 10-ns MD simulation as
284 computed by *GROMACS* (48), with the Amber99SB and GAFF force fields (49,50). Uncertainty is the sample
285 standard deviation.
286
287 [d] Local distance difference test metric to assess confidence for structures predicted by *Colabfold* (44), averaged over
288 all residues. Uncertainty is the sample standard deviation.
289
290 [e] Reference superposition values provided by comparing a second structure of *Gs* MutY in complex with its
291 transition state analog (PDB ID 6u7t)
292

293 We performed virtual docking experiments to examine the potential for molecular
294 interaction with adenosine and OG ligands. MutY scans DNA looking for the OG:A base pair by
295 sensing the major-groove disposition of the exocyclic amine of the OG base in its *syn*
296 conformation (51,52). After this initial encounter, the enzyme bends the DNA, flips the adenine
297 base from the DNA double helix into the active site pocket, and positions OG in its *anti*
298 conformation as seen in structures of the enzyme complexed to DNA (30,31). Thus, multiple
299 conformations and orientations for the OG and adenosine ligands were anticipated. The search
300 volume for the adenosine ligand was centered on the active site in the NTD, and the search
301 volume for the OG ligand was defined by the OG-recognition motif found in the CTD (see
302 **Materials and methods**). Representative outcomes obtained with *Autodock VINA* are shown in
303 **Fig 7,** and the corresponding binding affinities are reported in **Table 3** and Supporting
304 Information **Table S6**. As anticipated the precise orientation and position for these docked
305 ligands varied, and none exactly match the disposition of the adenine or OG base as presented in
306 the context of double stranded DNA. Nevertheless, binding affinities for the ligand-LCHF MutY
307 complexes ranged from -6.8 to -8.0 kcal/mol, indicating favorable interactions were attainable
308 and similar to the binding affinities measured for *Gs* MutY by the same virtual docking method.

309 Molecular dynamics simulations

310 Virtual docking is fast and computationally economical but largely ignores motion and
311 solvent. The reliability of docking improves when complemented with molecular dynamic (MD)
312 simulation (53,54). To further assess stability and dynamic properties of LCHF MutY-ligand
313 complexes derived by docking, we applied MD simulations with the *Amber* force field (49,50),
314 as implemented with *GROMACS* (48). Each protein-ligand complex was solvated in water,
315 charges were balanced with counterions, and the system was equilibrated in preparation for a 10-

316 ns MD simulation (see **Materials and methods**). Supporting Information **Fig S7** summarizes the
317 resulting trajectories in terms of energy, distance and structure over time. We focused on
318 mechanistically relevant interactions by tracking distances from the base moiety to the catalytic
319 Glu residue for adenosine complexes, and distances to the OG-recognition Ser/Thr residue for
320 OG complexes. MD trajectories for the *Gs* MutY-ligand complexes (**Fig S7A** and **Fig S7B**)
321 provided a basis of comparison for the LCHF MutY-ligand complexes.

322 All MutY-ligand complexes were characterized by favorable binding affinity, extracted
323 as the sum of local Lennard-Jones and Coulombic interaction terms averaged over the 10-ns MD
324 simulation (**Table 3**). While all ligands were mobile, adjusting position and orientation over time
325 with respect to the starting structure derived from virtual docking, the MD outcomes separated
326 into two groups distinguished by the degree of ligand movement. In the first group the adenosine
327 and OG ligands remained close to the original binding sites and engaged with functionally
328 important residues, sometimes via water-mediated contacts, throughout the 10-ns MD
329 simulation. This first group with persistently engaged ligands included the complexes with *Gs*
330 MutY (**Fig S7A** and **Fig S7B**), *Thiotrichaceae* MutY (**Fig S7G** and **Fig S7H**) and
331 *Flavobacteriaceae* MutY (**Fig S7I** and **Fig S7J**).

332 For example, the catalytic Glu46 of *Thiotrichaceae* MutY hydrogen bonded with N6 of
333 adenosine and with N7 via a bridging solvent molecule, with these interactions persisting for the
334 entire 10-ns simulation (**Fig S7G**). Water-mediated interaction of the catalytic Glu and N7
335 featured in MD simulations of *Gs* MutY complexed to double stranded DNA by others (55), and
336 was originally observed in the first crystal structure of *Gs* MutY complexed to substrate DNA
337 (30). Similarly, OG remained continuously bound at the interface of the NTD and CTD in its
338 complex with *Thiotrichaceae* MutY and with *Flavobacteriaceae* MutY. In the former, Ser306

339 engaged the OG ligand via hydrogen bonds to N1, N2 and O6 of the Watson-Crick-Franklin face
340 throughout the 10-ns simulation (**Fig S7H**). Interactions with the Watson-Crick-Franklin face of
341 OG, especially with N2 presented in the major groove, are known to facilitate initial recognition
342 of the OG lesion (51,52). Crystal structures feature the corresponding Ser of *Gs* MutY hydrogen
343 bonded with N7 and O8 of OG (30–32), and similar contacts between Ser305 and N7, O8 and
344 N6 of the Hoogsteen face are observed throughout the simulation of *Flavobacteriaceae* MutY
345 complexed to OG (**Fig S7J**).

346 By contrast with these persistently engaged ligands observed in the first group, ligands in
347 the second group disengaged and departed from the original binding site and found new sites as
348 observed for complexes with *Marinosulfonomonas* MutY (**Fig S7C** and **Fig S7D**) and
349 *Rhodobacteraceae* MutY (**Fig S7E** and **Fig S7F**). For example, during the *Marinosulfonomonas*
350 MutY simulation, adenosine slipped out of the active site pocket within 1 ns, remained near the
351 active site entrance until 6 ns, when it finally exited completely and nestled into a shallow
352 concavity on the outer surface of the enzyme defined by the loop containing Ser24 and a helix
353 from Ala58 to His65 (**Fig S7C**). The situation was comparable for adenosine complexed to
354 *Rhodobacteraceae* MutY, but the ligand found a different resting place after departing the active
355 site pocket (**Fig S7E**), wedged into a groove with residues Gly126 and Tyr128 on one side and
356 Gln49 and Arg93 on the other side. This alternate adenosine binding site for *Rhodobacteraceae*
357 MutY is adjacent and partially overlapping with the exosite observed for cytosine in the complex
358 of *Gs* MutY with its OG:C anti-substrate (56). Departure of the base from the active site as
359 observed in our MD simulations was anticipated since crystal structures of MutY in complex
360 with enzyme-generated abasic site (AP) product show no electron density for the base moiety
361 (34), implying that the free base has an escape route.

362 Binding site departure was also observed for the OG ligand, which disengaged
363 precipitously and found a new binding site making contact with Gln54, which caps one helix,
364 and Tyr94, which caps a neighboring alpha helix on the surface of the NTD of
365 *Marinosulfonomonas* MutY (**Fig S7D**). This MD simulation for the *Marinosulfonomonas* MutY-
366 OG complex also featured large scale movement of the CTD relative to the NTD (**Fig S7D**), an
367 event not seen for the other OG complexes. At the outset, the OG ligand bound to
368 *Rhodobacteraceae* MutY with OG-specific hydrogen bonds connecting Thr299, N7 and O8
369 atoms (**Fig S7F**), very comparable to hydrogen bonds seen in the crystal structure of *Gs* MutY
370 bound to DNA with the OG lesion (30–32). However, the FTH loop of *Rhodobacteraceae* MutY
371 pulled away early in the MD simulation, thereby breaking these hydrogen bonds, and the OG
372 ligand adopted a new orientation while remaining wedged between the NTD and CTD.
373 In summary, MD simulations confirmed thermodynamic stability for the MutY-ligand
374 complexes predicted by molecular docking and differentiated these into two groups based on
375 conformational flexibility. The ultra-stable ligand complexes which persisted throughout the 10-
376 ns MD simulation included those with predicted structures for two of the LCHF MutYs as well
377 as the ligand complexes with the x-ray crystal structure of *Gs* MutY (PDB ID 6u7t). Kinetically
378 unstable ligand complexes observed for the other two LCHF MutYs prompted further *in vivo*
379 validation for the LCHF MutYs to address the open question, which enzyme, if any, could
380 support function?

381 Testing mutation suppression activity of LCHF MutY enzymes by 382 recombinant expression

383 The *in silico* experiments provided strong evidence that the LCHF MutYs are structurally
384 comparable to authentic MutY enzymes, with affinity for OG:A lesions, suggesting these also

385 function to prevent mutations. To demonstrate biological function directly, we recombinantly
386 expressed the genes in *E. coli* and measured mutation suppression activity *in vivo*. Three of the
387 representative LCHF MutYs were successfully cloned into the pKK223 expression plasmid as
388 verified by Sanger sequencing. The *Flavobacteriaceae* MutY appeared to be toxic to *E. coli* as
389 only mutant versions of the gene were obtained from multiple cloning attempts, a situation that is
390 reminiscent of *Gs* MutY, which is also apparently toxic to *E. coli* and could not be cloned into
391 pKK223 (33).

392 To test the mutation suppression activity of *Marinosulfonomonas, Rhodobacteraceae,*
393 *and Thiotrichaceae* MutYs, we measured mutation rates with a rifampicin resistance assay (57).
394 Several, independent, single-point mutations within the gene encoding RNA polymerase beta-
395 subunit (*rpoB*) confer antibiotic resistance (58,59). Thus, spontaneous rifampicin resistant (Rif^R)
396 mutants arising in overnight cultures can be counted by the colonies that emerge on rifampicin
397 containing plates. Cultures expressing functional MutY delivered by plasmid DNA
398 transformation have low Rif^R frequency compared to the high Rif^R frequency characterizing the
399 reporter strain that lacks *mutY* and *mutM* genes (see **Materials and methods**).

400 Cultures with an empty plasmid (*null*) and cultures with a plasmid encoding *Ec* MutY
401 showed significant differences in the frequency of Rif^R mutants, with median values of 101 and
402 12, respectively, indicating the assay was fit for use (significance determined by non-overlap of
403 median 95% confidence intervals) (**Fig 8** and Supporting Information **Table S8**).
404 *Marinosulfonomonas* MutY*, Rhodobacteraceae* MutY*,* and *Thiotrichaceae* MutY each
405 demonstrated significant mutation suppression activity when compared to the *null*. Indeed,
406 *Rhodobacteraceae* MutY showed mutation suppression performance equivalent to that measured
407 for *Ec* MutY, and *Marinosulfonomonas* MutY was apparently better at suppressing mutations

408 than *Ec* MutY (**Fig 8**), a remarkable outcome given the evolutionary time separating these

409 species. *Thiotrichaceae* MutY showed partial function. Cultures expressing *Thiotrichaceae*

410 MutY suppressed Rif^R mutants to 50% of the rate observed for *null* cultures, but allowed a

411 mutation rate about 4-fold greater than that measured for cultures expressing *Ec* MutY (**Fig 8**

412 and Supporting Information **Table S8**).

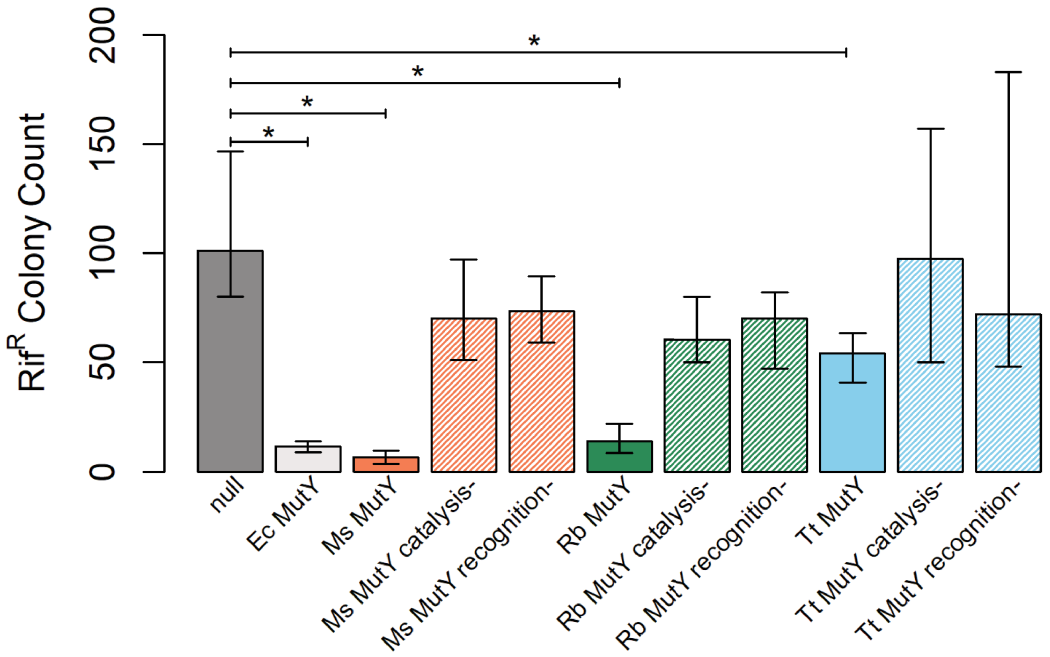

Fig 8. **Functional analysis.** Bars represent median Rif^R colony counts for *E. coli* cultures expressing MutY, MutY variants, or no MutY (*null*) from a plasmid DNA. Error bars represent 95% confidence intervals as determined by bootstrap sampling (see Supporting Information **Table S8** for tabulated values). *Marinosulfonomonas* (*Ms*) MutY, *Rhodobacteraceae* (*Rb*) MutY, and *Thiotrichaceae* (*Tt*) MutY each suppressed mutations as evidenced by non-overlap of Rif^R confidence intervals compared to *null* cultures. Altered versions of each LCHF MutY tested the importance of residues for catalysis and OG-recognition. Designated *catalysis-* and *recognition-* along the X-axis, these alterations severely impacted mutation suppression function indicating the LCHF MutYs share mechanistic features with the extensively studied enzymes *Ec* MutY and *Gs* MutY.

413

414 To investigate the biochemical mechanism employed by LCHF MutY enzymes, we

415 altered residues essential for OG:A recognition and catalysis, then repeated the mutation

416 suppression assay. Two mutants of each LCHF MutY were constructed through site-directed

417 substitution of residues. One set of substitutions was designed to disable the OG-recognition
418 motif by replacing F(S/T)H residues (**Fig 2** and **Fig 5**) with alanine residues (designated
419 *recognition*-); the other set of substitutions was designed to disable catalysis by replacing the
420 active site Asp and Glu residues with structurally similar, but chemically inert Asn and Gln
421 residues (*catalysis*-). For all three LCHF MutYs, these targeted substitutions disabled mutation
422 suppression function *in vivo* as shown by elevated Rif^R frequencies for cultures expressing the
423 *recognition*- and *catalysis*- versions. The mutation frequencies for cultures expressing these site-
424 specific substitution variants were comparable to the Rif^R frequencies measured for *null* cultures
425 as judged by overlapping 95% confidence intervals (**Fig 8** and Supporting Information **Table**
426 **S8**). These results indicate that the LCHF MutYs suppress mutations by a mechanism that is
427 highly similar to the strategy executed by *Ec* MutY and *Gs* MutY.
428

429 # Discussion

430 To gain insight into DNA repair strategies in early Earth-like environments, we
431 investigated the status of the GO DNA repair system within microbes inhabiting the LCHF. Our
432 approach included mining of metagenomic data, bioinformatic comparisons informed by
433 structure and mechanistic understanding, predictive molecular modeling, and functional
434 analyses. The degree to which this approach succeeded was dependent on the assembly of
435 metagenomic sequences into contigs long enough to contain full-length genes (35). Earlier
436 attempts to search for MutY genes within previous LCHF metagenomes with shorter contigs
437 yielded a number of hits, but these were truncated and therefore missing critical motifs,
438 explaining weak mutation suppression function (unpublished results). The longer contigs utilized
439 in this study allowed us to capture entire MutY genes, bin these MutY-encoding contigs into

440 MAGs to assess associated gene inventories, and thereby infer metabolic strategies for the
441 microbes expressing the GO DNA repair components.

442 Within the initial set of 649 LCHF MutY candidates identified by sequence identity, 160
443 genes encoded proteins with all of the chemical motifs known to be important for MutY
444 function. Indeed, leveraging the extensive body of knowledge obtained from crystal structures
445 and mechanistic studies allowed us to select features such as sequence length, presence of MutY
446 motifs, and structural prediction to distinguish LCHF MutYs from other members of the helix-
447 hairpin-helix (HhH) superfamily. Recombinant expression in *E. coli* revealed that LCHF MutY
448 representatives suppress mutations *in vivo* by a mechanism that depends on the catalytic and OG-
449 recognition motifs (**Fig 8**), strongly suggesting these are functional enzymes that actively seek
450 and initiate repair of OG:A lesions within their respective LCHF microbes. Toxicity observed for
451 one LCHF gene encoding *Flavobacteriaceae* MutY, which could not be cloned except with
452 disabling nonsense mutations, underscores the risks and dangers posed by MutY and DNA
453 glycosylases in general, which initiate DNA repair by damaging the DNA further, creating AP
454 sites that are themselves destabilizing (60). The potential for lethal outcomes makes cross-
455 species function observed for *Marinosulfonomonas* MutY and *Rhodobacteraceae* MutY across
456 vast evolutionary time all the more remarkable.

457 Retained function across evolutionary and species barriers strongly suggests that MutY
458 interacts with the base excision repair apparatus through some well-preserved mechanism that
459 relies on a universal language understood by all organisms. Most critically, the AP sites
460 generated by MutY should be recognizable to downstream AP nucleases. Protein-protein
461 interactions between AP nucleases and MutY have been discussed as a possible mechanism (61–
462 63), but on its own such a mechanism would rely on coevolution of protein partners. Our results

463 and those reported by others for complementation with the eukaryote homolog MUTYH (64)
464 speaks for a mechanism that is less sensitive to sequence divergence. Therefore, we favor a
465 model where the distorted DNA structure created by MutY signals the location of the AP site for
466 handoff to the BER apparatus, as has been suggested previously (62).

467 *Thiotrichaceae* MutY underperformed in our functional evaluation, despite coming from
468 a gammaproteobacterium most closely related to the *E. coli* employed for the bioassay. Lower
469 mutation suppression performance observed for *Thiotrichaceae* MutY may simply be due to
470 differences in conditions for our *in vivo* experiments and more extreme conditions found in the
471 habitat where *Thiotrichaceae* thrives at the LCHF. In support of this adaptation to extreme
472 environments idea, the LCHF enzymes which were predicted to have the highest stability and
473 form the most stable ligand complexes that persisted throughout MD simulations appeared
474 incompatible with mesophile biology, being either apparently lethal (*Flavobacteriaceae* MutY)
475 or relatively ineffective at suppressing mutations (*Thiotrichaceae* MutY). This pattern of
476 predicted high stability incompatible with mesophile biology extends also to the reference
477 enzyme *Gs* MutY which is from a known thermophile and also appeared lethal in the reporter
478 bacterium, necessitating a chimera approach for evaluation of biological function (33). Adapted
479 for stability at higher temperatures, these enzymes may lack flexibility needed to perform their
480 catalytic duty at lower temperatures, an idea described previously as "corresponding states" of
481 conformational flexibility (65–67). In future work, biochemical characterization of purified
482 LCHF MutY enzymes at high temperatures could address this model directly.

483 Our metagenomic analysis revealed that gene homologs encoding the GO DNA repair
484 system are abundant in basement microbes inhabiting the LCHF (**Fig 3**). This observation is
485 surprising given that the basement of the LCHF is expected to be anoxic (1,3). The chemical

486 agents commonly thought of for producing oxidized guanine (OG) are ROS derived from
487 molecular oxygen *via* aerobic metabolism. In an anoxic environment, what chemical agents are
488 producing OG and how are these generated? Models of hydrothermal field chemistry predict
489 abiotic production of ROS which the microbial residents may encounter, although these would
490 probably react with cell protective structures before encountering DNA (68). Continual mixing
491 of seawater with the anoxic hydrothermal fluid could provide molecular oxygen at the interface
492 where hydrothermal fluids vent into ambient seawater at the seafloor (1,3). Facultative anaerobes
493 at this interface would inevitably generate ROS (69,70), and therefore benefit from the GO DNA
494 repair system. Intermicrobial competition has driven acquisition of chemical strategies, including
495 ROS, for killing other bacteria (71–73), but there is currently no evidence for such bacterial
496 warfare in basement dwelling microbes of the LCHF.

497 Another explanation for the source of OG in basement microbes of the LCHF, which is
498 suggested by our gene inventory analysis (**Table 2** and Supporting information **Table S4**),
499 involves reactive sulfur species (RSS) and reactive nitrogen species (RNS). Many of the
500 basement-dwelling microbes within the LCHF appear to metabolize sulfur and nitrogen for
501 energy conservation (4,35), strategies that generate RSS and RNS as metabolic byproducts
502 (74,75). Indeed, mechanisms for the oxidation of guanine by both RSS and RNS have been
503 described, including the formation of 8-oxoguanine (OG) and chemically similar 8-nitroguanine,
504 which templates for adenine in a fashion similar to OG (76–78). The oxidation of guanine by
505 RSS and RNS generated from microbial metabolism would produce OG independent of
506 molecular oxygen and thereby necessitate the GO DNA repair system for both facultative and
507 obligate anaerobes inhabiting the LCHF.

508 Whether organisms developed biochemical systems to deal with oxidative damage before
509 or after the Great Oxidation Event (GOE) remains an open question (79,80). It is reasonable to
510 think of these systems arising in response to the selective pressure of oxidative damage from
511 rising O_2 levels. However, it is also possible that these systems were already in place as a coping
512 mechanism for other oxidants and were repurposed to deal with the new source of oxidizing
513 agents when O_2 became readily available. Indeed, obligate anaerobes contain many of the same
514 pathways to deal with oxidative damage as aerobes (79,80). Our discovery of the GO DNA
515 repair system in basement dwellers of the LCHF adds to this body of evidence and supports the
516 hypothesis that oxidative damage repair systems were established before the GOE. We
517 considered the caveat of possible phage-mediated gene transfer – modern microbes adapted to
518 oxygen-rich regions elsewhere may be the source of LCHF MutYs. However, the
519 correspondence of taxonomic assignments based on MutY sequence and based on MAGs and the
520 high degree of sequence diversity seen for LCHF MutY enzymes are inconsistent with expansion
521 of GO DNA repair in the LCHF by horizontal gene transfer. Thus, it seems more likely that
522 LCHF microbes inherited the GO DNA repair system from a common ancestor and retained it
523 through necessity, even in the absence of extrinsic O_2.
524

525 # Conclusion

526 Performing empirical studies on how life may have evolved on Earth and other planets is
527 inherently difficult due to time and spatial barriers. Unique sites such as the LCHF serve as
528 representatives of these theorized environments (3,81). By discovering the GO DNA repair
529 system at the LCHF and validating mutation suppression function by LCHF MutYs, we infer that
530 microbes within the anoxic environment of the LCHF basement are under evolutionary pressure

531 to repair OG lesions. Evolutionary pressure and the source of OG appear to be driven by nitrogen
532 reactive species or sulfur reactive species as supported by the metabolic survey of the MutY-
533 encoding organisms. These results highlight the need for DNA-based life to manage oxidized
534 guanine damage even in anoxic environments. Moreover, this work adds evidence for the more
535 general hypothesis that life established biochemical systems to deal with oxidative damage early,
536 well before the GOE, and should be considered when developing an evolutionary model for early
537 life.
538

539 Materials and Methods

540 Metagenomic sequencing and analysis of LCHF fluid samples

541 Generation, assembly, and annotation of metagenomes from the Lost City Hydrothermal
542 Field (LCHF) have been described previously (35), and are briefly summarized here. In 2018,
543 the remotely operated vehicle (ROV) Jason collected samples of fluids venting from chimneys at
544 the LCHF, which is located near the Mid-Atlantic Ridge at 30 ºN latitude and a depth of ~800 m.
545 Whole-genome community sequences ("metagenomes") were generated from the fluid samples,
546 and assembled metagenomic contigs were binned into metagenome-assembled genomes
547 (MAGs). Potential gene homologs encoding enzymes involved in the GO DNA repair system
548 were identified by conducting KEGG (82,83) orthology assignment using the *BlastKOALA* v2.2
549 program (84). The selected genes that were identified include: *mutT* (KEGG ID: K03574), *mutM*
550 (K10563), and *mutY* (K03575) along with the genes *xthA* (K01142) and *nfo* (K01151), which
551 encode exonuclease III and endonuclease IV, respectively.
552 The relative abundance of each GO repair pathway gene homolog at each LCHF chimney

553 location was calculated as the normalized metagenomic sequence coverage, determined by
554 mapping of reads from each fluid sample against the pooled assembly. Coverages are reported as
555 transcripts (or metagenomic fragments) per million (TPM), which is a proportional unit suitable
556 for comparisons of relative abundances between samples (35,85).

557 Identification of LCHF MutYs

558 Candidate MutY genes in the LCHF metagenomes were identified with a *BLASTP* search
559 against predicted protein sequences from the LCHF pooled metagenomic assembly using MutY
560 queries from *Gs* MutY (NCBI Accession ID: P83847.2) and *Ec* MutY (NCBI Accession ID:
561 CDP76921.1). The diversity of these candidates, visualized by aligning the sequences along with
562 *Gs* MutY and *Ec* MutY with *Clustal Omega* (86), and an initial phylogeny was built with *iTOL*
563 (87). LCHF MutY candidate sequences were then aligned by *PROMALS3D* (88), guided by the
564 structure of *Gs* MutY (PDB ID 6u7t). Sequence diversity in the C-terminal domain prevented
565 reliable alignment of this region in this first pass at structure-guided alignment. To overcome this
566 challenge, sequences were split into two parts: one part with all residues before position Val147
567 in the *Gs* MutY protein which were reliably aligned, and the second part with all residues
568 following position Asn146 which were aligned inconsistently in the first pass. These two parts
569 were separately resubmitted for alignment by *PROMALS3D* guided by the corresponding
570 portions of the crystal structure. For inclusion in this alignment, the C-terminal part was required
571 to pass a minimum length criteria of 160 residues. The resulting alignment was inspected for the
572 MutY-defining chemical motifs described in the text, and a phylogeny was constructed for the
573 160 authenticated LCHF MutYs with *iTOL* (87). Selection of the four representative LCHF
574 MutYs was guided by this phylogeny and by the completeness of each associated MAG.

575 ## Taxonomic classification

576 Contiguous DNA sequences containing the LCHF MutY representatives were assigned
577 taxonomic classifications using the program *MMseqs2* (89) and the Genome Taxonomy
578 Database (GTDB) as described previously (35). Taxonomic classification of each MAG that
579 included a contig of interest was performed with *GTDB-Tk* v1.5.1 (90). The environmental
580 distributions of MutY-encoding MAGs were inspected for potential signs of contamination from
581 ambient seawater. This possibility was ruled out by the absence of all MutY-encoding taxa
582 reported in this study in the background seawater samples. MAG completeness and
583 contamination scores were generated by *CheckM* v1.0.5 as described previously (35).

584 ## Prediction of physical parameters

585 The theoretical molecular weights and pIs of the LCHF MutY representatives and known
586 MutY sequences were generated with ExPASY (91). The theoretical melting temperature of the
587 representatives was calculated with the Tm predictor from the Institute of Bioinformatics and
588 Structural Biology, National Tsing-Hua University (92).

589 ## Molecular modeling

590 Protein structures for the LCHF MutY representatives were predicted by Colabfold with
591 use of MMseqs2 alignments and relaxed with the Amber force field (44,89,93,94). Predicted
592 structures were superimposed with the crystal structure of *Gs* MutY (PDB ID 3g0q) to generate
593 RMSD (Å) for pruned atom pairs using the MatchMaker tool in *ChimeraX* (45). Initial
594 superpositions were dominated by residues in the N-terminal domain. To fairly compare
595 structures for the more diverse C-terminal domains, the linker region between domains was

596 identified by inspection, and superposition with *Gs* MutY was repeated with selection of residues
597 in the N-terminal domain and, separately, in the C-terminal domain.
598 Ligand docking experiments were executed with the program *AutoDock VINA* (46,47).
599 Ligand structures representing adenosine and OG were prepared with the ligand preparation tools
600 implemented with *Autodock Tools* (95,96). Receptor structures were prepared from the structures
601 predicted by Colabfold or from the crystal structure of *Gs* MutY (PDB ID 6u7t), each after
602 superposition with PDB ID 3g0q, with the receptor preparation tools as implemented with
603 *Autodock Tools* (95,96). Receptor structures were treated as rigid objects, and ligands included
604 two active torsion angles defined by the C1'-N9 and C4'-C5' bonds. Separate 24 x 24 x 24 $Å^3$
605 search volumes were defined for adenosine and for OG. The adenosine search volume was
606 centered on the position of atom C1' in the residue A5L:18 in chain C of the *Gs* MutY crystal
607 structure (PDB ID 3g0q), and the OG search volume was centered on the position of atom C1' in
608 residue 8OG:6 in chain B of the same structure.
609 MD simulations were performed with *GROMACS* version 2022.5 (48), applying the
610 AMBER99SB and GAFF force fields (49,50), with CPU and GPU nodes at the University of
611 Utah's Center for High Performance Computing. We followed steps outlined in the *GROMACS*
612 tutorial "Protein-Ligand Complex" as a guide for our experiments (97). The starting structure for
613 a protein-ligand complex was selected from the binding modes predicted by *Autodock VINA*,
614 choosing the mode with the highest affinity after excluding those that appeared incompatible
615 with the double stranded DNA-enzyme structure. To conserve computational resources,
616 simulation of the complex with adenosine was limited to N-terminal residues as follows: residues
617 8-220 for *Gs* MutY (PDB ID 6u7t); 6-230 for *Marinosulfonomonas* MutY; 2-220 for
618 *Rhodobacteraceae* MutY; 11-223 for *Thiotrichaceae* MutY; and 2-209 for *Flavobacteriaceae*

619 MutY. Simulations with OG omitted the iron-sulfur cluster domain and interdomain linker and
620 thus included limited residues as follows: residues 29-137, 234-289, 295-360 for *Gs* MutY; 40-
621 142, 239-352 for *Marinosulfonomonas* MutY; 38-139, 233-352 for *Rhodobacteraceae* MutY;
622 32-140, 238-364 for *Thiotrichaceae* MutY; and 19-127, 234-354 for *Flavobacteriaceae* MutY.
623 Ligand topology files were generated with the *ACPYPE* server (98), applying the general *Amber*
624 force field (49). Each complex was solvated with water molecules with three points of
625 transferable intermolecular potential (TIP3P). Counterions were added to neutralize the net
626 charge of the system. The system was energy minimized by 50000 steepest descent steps and
627 further equilibrated in two phases, NVT followed by NPT, each entailing 100 ps with 2-fs steps.
628 Temperature coupling during NVT and NPT equilibration was accomplished with a modified
629 Berendsen thermostat set to the reference temperature 300 K. Pressure coupling during NPT
630 equilibration was accomplished with the Berendsen algorithm set to the reference pressure 1 bar.
631 The equilibrated system was subjected to a 10-ns MD production run with 2-fs steps, applying a
632 modified Berendsen thermostat (300 K reference temperature) and Parrinello-Rahman barostat (1
633 bar reference pressure). Short range interaction energies and distances were extracted from the
634 resulting trajectories with use of *GROMACS* functions and plotted with the *R* package *ggplot2*
635 (99).

636 Recombinant DNA cloning

637 Synthetic genes encoding the LCHF MutYs were codon optimized for expression in *E.*
638 *coli* except that pause sites with rare codons were engineered so as to retain pause sites found in
639 the gene encoding *Ec* MutY. GBlocks gene fragments were ordered from Integrated DNA
640 Technologies. LCHF MutY genes designed in this way were cloned into the low-expression
641 pKK223 vector by ligation-independent cloning (LIC). PCR reactions with the high-fidelity

642 Phusion polymerase (Agilent) amplified the synthetic gBlock and two overlapping fragments
643 derived from approximate halves of the pKK223 plasmid. PCR products were separated by
644 electrophoresis in 0.8% agarose x1 TAE gels containing 1 µg/mL ethidium bromide. DNA was
645 visualized by long-wavelength UV shadowing to allow dissection of gel bands, and the DNA
646 was purified with the GeneJet gel extraction system (thermo scientific), treated with Dpn1 (New
647 England Biolabs) at 37 °C for 45 min, and heat shock transformed directly into DH5α competent
648 cells. Clones were selected on ampicillin media plates. The plasmid DNA was purified from 4-
649 mL overnight cultures by use of the Wizard Plus MiniPrep kit (Promega) according to the
650 manufacturer's instructions. The sequence of the LCHF MutY encoding gene was verified by
651 Sanger sequencing with UpTac and TacTerm primers. Genes encoding site-directed substitution
652 variants were created by amplifying two overlapping fragments of the LCHF MutY-pKK223
653 plasmid with mutagenic PCR primers followed by similar gel purification and transformation
654 procedures. In our hands the LIC cloning efficiency was close to 95% except for the
655 *Flavobacteriaceae* MutY gene which could not be cloned intact. The pkk223 plasmids
656 containing the LCHF MutY encoding genes can be found on Addgene with ID numbers 210791-
657 210799.

658 Mutation suppression assay

659 Mutation rates were measured by the method outlined previously (33,57). The CC104
660 *mutm::KAN muty::TET* cells (100) were heat-shock transformed with a pKK223 plasmid
661 encoding the *Ec* MutY gene, LCHF MutY genes, or no gene (*null*). Transformants selected from
662 kanamycin-ampicillin-tetracycline (KAT) media plates were diluted prior to inoculation of 2-mL
663 KAT liquid media, and these cultures were grown overnight for 18 hours at 37°C with shaking at
664 180 rpm. Cultures were kept cold on ice or at +4 °C prior to further processing. Cells were

665 collected by centrifugation, the media was removed by aspiration, and cells were resuspended in
666 an equal volume of 0.85% sodium chloride before seeding 100 µL aliquots to kanamycin-
667 ampicillin-rifampicin (KAR) media plates. Dilutions of the washed cells were also seeded to
668 KAR plates (10^{-1} dilution) and kanamycin-ampicillin (KA) plates (10^{-7} dilution), and allowed to
669 overnight at 37°C for 18 hours. The number of Rif^R mutants were counted by counting the
670 colony forming units (CFU). Statistical analysis was performed in *R* as previously described
671 (33). Confidence intervals were obtained by bootstrap resampling of 10,000 trials as
672 implemented in *R* with the *boot* package (101,102).
673

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References

1. Kelley DS, Karson JA, Früh-Green GL, Yoerger DR, Shank TM, Butterfield DA, et al. A Serpentinite-Hosted Ecosystem: The Lost City Hydrothermal Field. Science. 2005 Mar 4;307(5714):1428–34.
2. Amador ES, Bandfield JL, Brazelton WJ, Kelley D. The Lost City Hydrothermal Field: A Spectroscopic and Astrobiological Analogue for Nili Fossae, Mars. Astrobiology. 2017 Nov;17(11):1138–60.
3. Kelley DS, Karson JA, Blackman DK, Früh-Green GL, Butterfield DA, Lilley MD, et al. An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. Nature. 2001 Jul;412(6843):145–9.
4. Brazelton WJ, Schrenk MO, Kelley DS, Baross JA. Methane- and Sulfur-Metabolizing Microbial Communities Dominate the Lost City Hydrothermal Field Ecosystem. Appl Environ Microbiol. 2006 Sep;72(9):6257–70.
5. Russell MJ, Hall AJ, Martin W. Serpentinization as a source of energy at the origin of life. Geobiology. 2010 Dec;8(5):355–71.
6. Proskurowski G, Lilley MD, Seewald JS, Früh-Green GL, Olson EJ, Lupton JE, et al. Abiogenic hydrocarbon production at lost city hydrothermal field. Science. 2008 Feb 1;319(5863):604–7.
7. Lang SQ, Butterfield DA, Lilley MD, Paul Johnson H, Hedges JI. Dissolved organic carbon in ridge-axis and ridge-flank hydrothermal systems. Geochim Cosmochim Acta [Internet]. 2006 Aug [cited 2023 Nov 28];70(15). Available from: https://par.nsf.gov/biblio/10088640-dissolved-organic-carbon-ridge-axis-ridge-flank-hydrothermal-systems
8. Lang SQ, Brazelton WJ. Habitability of the marine serpentinite subsurface: a case study of the Lost City hydrothermal field. Philos Trans R Soc Math Phys Eng Sci. 2020 Feb 21;378(2165):20180429.
9. Bruskov VI, Malakhova LV, Masalimov ZK, Chernikov AV. Heat-induced formation of reactive oxygen species and 8-oxoguanine, a biomarker of damage to DNA. Nucleic Acids Res. 2002 Mar 15;30(6):1354–63.
10. Kino K, Hirao-Suzuki M, Morikawa M, Sakaga A, Miyazawa H. Generation, repair and replication of guanine oxidation products. Genes Environ. 2017 Aug 1;39:21.
11. Nghiem Y, Cabrera M, Cupples CG, Miller JH. The mutY gene: a mutator locus in Escherichia coli that generates G.C----T.A transversions. Proc Natl Acad Sci. 1988 Apr;85(8):2709–13.

12. Cabrera M, Nghiem Y, Miller JH. mutM, a second mutator locus in Escherichia coli that generates G.C----T.A transversions. J Bacteriol. 1988 Nov;170(11):5405–7.
13. Maki H, Sekiguchi M. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. Nature. 1992 Jan;355(6357):273–5.
14. Michaels ML, Cruz C, Grollman AP, Miller JH. Evidence that MutY and MutM combine to prevent mutations by an oxidatively damaged form of guanine in DNA. Proc Natl Acad Sci U S A. 1992 Aug 1;89(15):7022–5.
15. Prakash A, Doublié S, Wallace SS. Chapter 4 - The Fpg/Nei Family of DNA Glycosylases: Substrates, Structures, and Search for Damage. In: Doetsch PW, editor. Progress in Molecular Biology and Translational Science [Internet]. Academic Press; 2012 [cited 2023 Mar 20]. p. 71–91. (Mechanisms of DNA Repair; vol. 110). Available from: https://www.sciencedirect.com/science/article/pii/B9780123876652000043
16. Denver DR, Swenson SL, Lynch M. An evolutionary analysis of the helix-hairpin-helix superfamily of DNA repair glycosylases. Mol Biol Evol. 2003 Oct;20(10):1603–11.
17. Trasviña-Arenas CH, Demir M, Lin WJ, David SS. Structure, function and evolution of the Helix-hairpin-Helix DNA glycosylase superfamily: Piecing together the evolutionary puzzle of DNA base damage repair mechanisms. DNA Repair. 2021 Dec 1;108:103231.
18. Kuwahara H, Takaki Y, Shimamura S, Yoshida T, Maeda T, Kunieda T, et al. Loss of genes for DNA recombination and repair in the reductive genome evolution of thioautotrophic symbionts of Calyptogena clams. BMC Evol Biol. 2011 Oct 3;11(1):285.
19. Garcia-Gonzalez A, Rivera-Rivera R, Massey S. The Presence of the DNA Repair Genes mutM, mutY, mutL, and mutS is Related to Proteome Size in Bacterial Genomes. Front Genet. 2012;3:3.
20. Jansson K, Blomberg A, Sunnerhagen P, Alm Rosenblad M. Evolutionary loss of 8-oxo-G repair components among eukaryotes. Genome Integr. 2010 Sep 1;1(1):12.
21. Fowler RG, Schaaper RM. The role of the mutT gene of Escherichia coli in maintaining replication fidelity. FEMS Microbiol Rev. 1997 Aug;21(1):43–54.
22. Boiteux S, O'Connor TR, Laval J. Formamidopyrimidine-DNA glycosylase of Escherichia coli: cloning and sequencing of the fpg structural gene and overproduction of the protein. EMBO J. 1987 Oct;6(10):3177–83.
23. Shida T, Noda M, Sekiguchi J. Cleavage of single- and double-stranded DNAs containing an abasic residue by Escherichia coli exonuclease III (AP endonuclease VI). Nucleic Acids Res. 1996 Nov 15;24(22):4572–6.
24. Parikh SS, Mol CD, Tainer JA. Base excision repair enzyme family portrait: integrating the structure and chemistry of an entire DNA repair pathway. Struct Lond Engl 1993. 1997 Dec 15;5(12):1543–50.
25. Ljungquist S, Lindahl T, Howard-Flanders P. Methyl methane sulfonate-sensitive mutant of Escherichia coli deficient in an endonuclease specific for apurinic sites in deoxyribonucleic acid. J Bacteriol. 1976 May;126(2):646–53.
26. Lind PA, Andersson DI. Whole-genome mutational biases in bacteria. Proc Natl Acad Sci. 2008 Nov 18;105(46):17878–83.
27. Foster PL, Lee H, Popodi E, Townes JP, Tang H. Determinants of spontaneous mutation in the bacterium *Escherichia coli* as revealed by whole-genome sequencing. Proc Natl Acad Sci [Internet]. 2015 Nov 3 [cited 2022 Aug 19];112(44). Available from: https://pnas.org/doi/full/10.1073/pnas.1512136112
28. Al-Tassan N, Chmiel NH, Maynard J, Fleming N, Livingston AL, Williams GT, et al.

773 Inherited variants of MYH associated with somatic G:C→T:A mutations in colorectal
774 tumors. Nat Genet. 2002;30(2):227–32.
775 29. Noll DM, Gogos A, Granek JA, Clarke ND. The C-Terminal Domain of the Adenine-DNA
776 Glycosylase MutY Confers Specificity for 8-Oxoguanine·Adenine Mispairs and May Have
777 Evolved from MutT, an 8-Oxo-dGTPase. Biochemistry. 1999 May 1;38(20):6374–9.
778 30. Fromme JC, Banerjee A, Huang SJ, Verdine GL. Structural basis for removal of adenine
779 mispaired with 8-oxoguanine by MutY adenine DNA glycosylase. Nature. 2004
780 Feb;427(6975):652–6.
781 31. Lee S, Verdine GL. Atomic substitution reveals the structural basis for substrate adenine
782 recognition and removal by adenine DNA glycosylase. Proc Natl Acad Sci. 2009 Nov
783 3;106(44):18497–502.
784 32. Woods RD, O'Shea VL, Chu A, Cao S, Richards JL, Horvath MP, et al. Structure and
785 stereochemistry of the base excision repair glycosylase MutY reveal a mechanism similar
786 to retaining glycosidases. Nucleic Acids Res. 2016 Jan 29;44(2):801–10.
787 33. Russelburg LP, O'Shea Murray VL, Demir M, Knutsen KR, Sehgal SL, Cao S, et al.
788 Structural Basis for Finding OG Lesions and Avoiding Undamaged G by the DNA
789 Glycosylase MutY. ACS Chem Biol. 2020 Jan 17;15(1):93–102.
790 34. Demir M, Russelburg LP, Lin WJ, Trasviña-Arenas CH, Huang B, Yuen PK, et al.
791 Structural snapshots of base excision by the cancer-associated variant MutY N146S reveal
792 a retaining mechanism. Nucleic Acids Res. 2023 Feb 22;51(3):1034–49.
793 35. Brazelton WJ, McGonigle JM, Motamedi S, Pendleton HL, Twing KI, Miller BC, et al.
794 Metabolic strategies shared by basement residents of the Lost City hydrothermal field
795 [Internet]. bioRxiv; 2022 [cited 2022 Apr 2]. p. 2022.01.25.477282. Available from:
796 https://www.biorxiv.org/content/10.1101/2022.01.25.477282v1
797 36. Porello SL, Leyes AE, David SS. Single-turnover and pre-steady-state kinetics of the
798 reaction of the adenine glycosylase MutY with mismatch-containing DNA substrates.
799 Biochemistry. 1998 Oct 20;37(42):14756–64.
800 37. Trasviña-Arenas CH, Lopez-Castillo LM, Sanchez-Sandoval E, Brieba LG. Dispensability
801 of the [4Fe-4S] cluster in novel homologues of adenine glycosylase MutY. FEBS J. 2016
802 Feb;283(3):521–40.
803 38. Schneider TD, Stephens RM. Sequence logos: a new way to display consensus sequences.
804 Nucleic Acids Res. 1990 Oct 25;18(20):6097–100.
805 39. Crooks GE, Hon G, Chandonia JM, Brenner SE. WebLogo: a sequence logo generator.
806 Genome Res. 2004 Jun;14(6):1188–90.
807 40. Gifford CM, Wallace SS. The genes encoding formamidopyrimidine and MutY DNA
808 glycosylases in Escherichia coli are transcribed as part of complex operons. J Bacteriol.
809 1999 Jul;181(14):4223–36.
810 41. Pomposiello PJ, Koutsolioutsou A, Carrasco D, Demple B. SoxRS-regulated expression
811 and genetic analysis of the yggX gene of Escherichia coli. J Bacteriol. 2003
812 Nov;185(22):6624–32.
813 42. Gralnick J, Downs D. Protection from superoxide damage associated with an increased
814 level of the YggX protein in Salmonella enterica. Proc Natl Acad Sci U S A. 2001 Jul
815 3;98(14):8030–5.
816 43. Gralnick JA, Downs DM. The YggX Protein of Salmonella enterica Is Involved in Fe(II)
817 Trafficking and Minimizes the DNA Damage Caused by Hydroxyl Radicals: Residue CYS-
818 7 is Essential for YggX Function. J Biol Chem. 2003 Jun 6;278(23):20708–15.

819 44. Mirdita M, Schütze K, Moriwaki Y, Heo L, Ovchinnikov S, Steinegger M. ColabFold:
820 making protein folding accessible to all. Nat Methods. 2022 Jun;19(6):679–82.
821 45. Meng EC, Goddard TD, Pettersen EF, Couch GS, Pearson ZJ, Morris JH, et al. UCSF
822 ChimeraX: Tools for structure building and analysis. Protein Sci Publ Protein Soc. 2023
823 Nov;32(11):e4792.
824 46. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a
825 new scoring function, efficient optimization and multithreading. J Comput Chem. 2010 Jan
826 30;31(2):455–61.
827 47. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: New Docking
828 Methods, Expanded Force Field, and Python Bindings. J Chem Inf Model. 2021 Aug
829 23;61(8):3891–8.
830 48. Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, Hess B, et al. GROMACS: High
831 performance molecular simulations through multi-level parallelism from laptops to
832 supercomputers. SoftwareX. 2015 Sep 1;1–2:19–25.
833 49. Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA. Development and testing of a
834 general amber force field. J Comput Chem. 2004 Jul 15;25(9):1157–74.
835 50. Hornak V, Abel R, Okur A, Strockbine B, Roitberg A, Simmerling C. Comparison of
836 multiple Amber force fields and development of improved protein backbone parameters.
837 Proteins. 2006 Nov 15;65(3):712–25.
838 51. Manlove AH, McKibbin PL, Doyle EL, Majumdar C, Hamm ML, David SS. Structure–
839 Activity Relationships Reveal Key Features of 8-Oxoguanine: A Mismatch Detection by
840 the MutY Glycosylase. ACS Chem Biol. 2017 Sep 15;12(9):2335–44.
841 52. Lee AJ, Majumdar C, Kathe SD, Van Ostrand RP, Vickery HR, Averill AM, et al.
842 Detection of OG:A Lesion Mispairs by MutY Relies on a Single His Residue and the 2-
843 Amino Group of 8-Oxoguanine. J Am Chem Soc. 2020 Aug 5;142(31):13283–7.
844 53. Liu X, Shi D, Zhou S, Liu H, Liu H, Yao X. Molecular dynamics simulations and novel
845 drug discovery. Expert Opin Drug Discov. 2018 Jan;13(1):23–37.
846 54. Guterres H, Im W. Improving Protein-Ligand Docking Results with High-Throughput
847 Molecular Dynamics Simulations. J Chem Inf Model. 2020 Apr 27;60(4):2189–98.
848 55. Nikkel DJ, Wetmore SD. Distinctive Formation of a DNA-Protein Cross-Link during the
849 Repair of DNA Oxidative Damage: Insights into Human Disease from MD Simulations
850 and QM/MM Calculations. J Am Chem Soc. 2023 Jun 21;145(24):13114–25.
851 56. Wang L, Lee SJ, Verdine GL. Structural Basis for Avoidance of Promutagenic DNA
852 Repair by MutY Adenine DNA Glycosylase. J Biol Chem. 2015 Jul 10;290(28):17096–
853 105.
854 57. Majumdar C, Nuñez NN, Raetz AG, Khuu C, David SS. Chapter Three - Cellular Assays
855 for Studying the Fe–S Cluster Containing Base Excision Repair Glycosylase MUTYH and
856 Homologs. In: David SS, editor. Methods in Enzymology [Internet]. Academic Press; 2018
857 [cited 2021 Jun 22]. p. 69–99. (Fe-S Cluster Enzymes Part B; vol. 599). Available from:
858 https://www.sciencedirect.com/science/article/pii/S0076687917303816
859 58. Garibyan L, Huang T, Kim M, Wolff E, Nguyen A, Nguyen T, et al. Use of the rpoB gene
860 to determine the specificity of base substitution mutations on the Escherichia coli
861 chromosome. DNA Repair. 2003 May 13;2(5):593–608.
862 59. Wu EY, Hilliker AK. Identification of Rifampicin Resistance Mutations in ***Escherichia***
863 ***coli***, Including an Unusual Deletion Mutation. J Mol Microbiol Biotechnol.
864 2017;27(6):356–62.

865 60. Loeb LA, Preston BD. Mutagenesis by apurinic/apyrimidinic sites. Annu Rev Genet.
866 1986;20:201–30.
867 61. Michaels ML, Tchou J, Grollman AP, Miller JH. A repair system for 8-oxo-7,8-
868 dihydrodeoxyguanine. Biochemistry. 1992 Nov 17;31(45):10964–8.
869 62. Pope MA, Porello SL, David SS. Escherichia coli apurinic-apyrimidinic endonucleases
870 enhance the turnover of the adenine glycosylase MutY with G:A substrates. J Biol Chem.
871 2002 Jun 21;277(25):22605–15.
872 63. Lu AL, Lee CY, Li L, Li X. Physical and functional interactions between Escherichia coli
873 MutY and endonuclease VIII. Biochem J. 2006 Jan 1;393(Pt 1):381–7.
874 64. Komine K, Shimodaira H, Takao M, Soeda H, Zhang X, Takahashi M, et al. Functional
875 Complementation Assay for 47 MUTYH Variants in a MutY-Disrupted Escherichia Coli
876 Strain. Hum Mutat. 2015 Jul;36(7):704–11.
877 65. Jaenicke R. Protein stability and molecular adaptation to extreme conditions. Eur J
878 Biochem. 1991 Dec 18;202(3):715–28.
879 66. Závodszky P, Kardos J, Svingor Á, Petsko GA. Adjustment of conformational flexibility is
880 a key event in the thermal adaptation of proteins. Proc Natl Acad Sci. 1998 Jun
881 23;95(13):7406–11.
882 67. Dong Y wei, Liao M ling, Meng X liang, Somero GN. Structural flexibility and protein
883 adaptation to temperature: Molecular dynamics analysis of malate dehydrogenases of
884 marine molluscs. Proc Natl Acad Sci. 2018 Feb 6;115(6):1274–9.
885 68. Shaw TJ, Luther GW, Rosas R, Oldham VE, Coffey NR, Ferry JL, et al. Fe-catalyzed
886 sulfide oxidation in hydrothermal plumes is a source of reactive oxygen species to the
887 ocean. Proc Natl Acad Sci U S A. 2021 Oct 5;118(40):e2026654118.
888 69. Messner KR, Imlay JA. The identification of primary sites of superoxide and hydrogen
889 peroxide formation in the aerobic respiratory chain and sulfite reductase complex of
890 Escherichia coli. J Biol Chem. 1999 Apr 9;274(15):10119–28.
891 70. Imlay JA. The molecular mechanisms and physiological consequences of oxidative stress:
892 lessons from a model bacterium. Nat Rev Microbiol. 2013 Jul;11(7):443.
893 71. Price RJ, Lee JS. Inhibition of Pseudomonas Species by Hydrogen Peroxide Producing
894 Lacobaccilli. J Food Prot. 1970 Jan 1;33(1):13–8.
895 72. Juven BJ, Pierson MD. Antibacterial Effects of Hydrogen Peroxide and Methods for Its
896 Detection and Quantitation†. J Food Prot. 1996 Nov 1;59(11):1233–41.
897 73. Bucci V, Nadell CD, Xavier JB. The Evolution of Bacteriocin Production in Bacterial
898 Biofilms. Am Nat. 2011 Dec;178(6):E162–73.
899 74. Rodionov DA, Dubchak IL, Arkin AP, Alm EJ, Gelfand MS. Dissimilatory Metabolism of
900 Nitrogen Oxides in Bacteria: Comparative Reconstruction of Transcriptional Networks.
901 PLOS Comput Biol. 2005 Oct 28;1(5):e55.
902 75. Han S, Li Y, Gao H. Generation and Physiology of Hydrogen Sulfide and Reactive Sulfur
903 Species in Bacteria. Antioxid Basel Switz. 2022 Dec 17;11(12):2487.
904 76. Suzuki N, Yasui M, Geacintov NE, Shafirovich V, Shibutani S. Miscoding events during
905 DNA synthesis past the nitration-damaged base 8-nitroguanine. Biochemistry. 2005 Jun
906 28;44(25):9238–45.
907 77. Joyner-Matos J, Predmore BL, Stein JR, Leeuwenburgh C, Julian D. Hydrogen Sulfide
908 Induces Oxidative Damage to RNA and DNA in a Sulfide-Tolerant Marine Invertebrate.
909 Physiol Biochem Zool PBZ. 2010;83(2):356–65.
910 78. Bhamra I, Compagnone-Post P, O'Neil IA, Iwanejko LA, Bates AD, Cosstick R. Base-

911 pairing preferences, physicochemical properties and mutational behaviour of the DNA
912 lesion 8-nitroguanine. Nucleic Acids Res. 2012 Nov;40(21):11126–38.
913 79. Ślesak I, Ślesak H, Kruk J. Oxygen and Hydrogen Peroxide in the Early Evolution of Life
914 on Earth: In silico Comparative Analysis of Biochemical Pathways. Astrobiology. 2012
915 Aug;12(8):775–84.
916 80. Lu Z, Imlay JA. When anaerobes encounter oxygen: mechanisms of oxygen toxicity,
917 tolerance and defence. Nat Rev Microbiol. 2021 Dec;19(12):774–85.
918 81. Shock EL, Schulte MD. Organic synthesis during fluid mixing in hydrothermal systems. J
919 Geophys Res Planets. 1998;103(E12):28513–27.
920 82. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids
921 Res. 2000 Jan 1;28(1):27–30.
922 83. Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for
923 taxonomy-based analysis of pathways and genomes. Nucleic Acids Res. 2023 Jan
924 6;51(D1):D587–92.
925 84. Kanehisa M, Sato Y, Morishima K. BlastKOALA and GhostKOALA: KEGG Tools for
926 Functional Characterization of Genome and Metagenome Sequences. J Mol Biol. 2016 Feb
927 22;428(4):726–31.
928 85. Thornton CN, Tanner WD, VanDerslice JA, Brazelton WJ. Localized effect of treated
929 wastewater effluent on the resistome of an urban watershed. GigaScience. 2020 Nov
930 19;9(11):giaa125.
931 86. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation
932 of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol.
933 2011 Jan;7(1):539.
934 87. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree
935 display and annotation. Nucleic Acids Res. 2021 Jul 2;49(W1):W293–6.
936 88. Pei J, Kim BH, Grishin NV. PROMALS3D: a tool for multiple protein sequence and
937 structure alignments. Nucleic Acids Res. 2008 Apr;36(7):2295–300.
938 89. Steinegger M, Söding J. MMseqs2 enables sensitive protein sequence searching for the
939 analysis of massive data sets. Nat Biotechnol. 2017 Nov;35(11):1026–8.
940 90. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify
941 genomes with the Genome Taxonomy Database. Bioinforma Oxf Engl. 2019 Nov
942 15;36(6):1925–7.
943 91. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: the
944 proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res. 2003
945 Jul 1;31(13):3784–8.
946 92. Ku T, Lu P, Chan C, Wang T, Lai S, Lyu P, et al. Predicting melting temperature directly
947 from protein sequences. Comput Biol Chem. 2009 Dec;33(6):445–50.
948 93. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate
949 protein structure prediction with AlphaFold. Nature. 2021 Aug;596(7873):583–9.
950 94. Eastman P, Swails J, Chodera JD, McGibbon RT, Zhao Y, Beauchamp KA, et al. OpenMM
951 7: Rapid development of high performance algorithms for molecular dynamics. PLoS
952 Comput Biol. 2017 Jul;13(7):e1005659.
953 95. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4
954 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput
955 Chem. 2009 Dec;30(16):2785–91.
956 96. Forli S, Huey R, Pique ME, Sanner M, Goodsell DS, Olson AJ. Computational protein-

ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc. 2016 May;11(5):905–19.
97. Lemkul JA. From Proteins to Perturbed Hamiltonians: A Suite of Tutorials for the GROMACS-2018 Molecular Simulation Package [Article v1.0]. Living J Comput Mol Sci. 2019;1(1):5068–5068.
98. Kagami L, Wilter A, Diaz A, Vranken W. The ACPYPE web server for small-molecule MD topology generation. Bioinformatics. 2023 Jun 1;39(6):btad350.
99. Wickham H. ggplot2: Elegant Graphics for Data Analysis [Internet]. New York, NY: Springer International Publishing; 2016 [cited 2023 Nov 27]. (Use R!). Available from: http://link.springer.com/10.1007/978-3-319-24277-4
100. Cupples CG, Miller JH. A set of lacZ mutations in Escherichia coli that allow rapid detection of each of the six base substitutions. Proc Natl Acad Sci U S A. 1989 Jul;86(14):5345–9.
101. Canty A, Ripley B. boot: Bootstrap Functions (Originally by Angelo Canty for S) [Internet]. 2022 [cited 2023 Nov 27]. Available from: https://cran.r-project.org/web/packages/boot/
102. Davison AC, Hinkley DV. Bootstrap Methods and their Application [Internet]. Cambridge: Cambridge University Press; 1997 [cited 2023 Nov 27]. (Cambridge Series in Statistical and Probabilistic Mathematics). Available from: https://www.cambridge.org/core/books/bootstrap-methods-and-their-application/ED2FD043579F27952363566DC09CBD6A
103. Osborne MJ, Siddiqui N, Landgraf D, Pomposiello PJ, Gehring K. The solution structure of the oxidative stress-related protein YggX from Escherichia coli. Protein Sci Publ Protein Soc. 2005 Jun;14(6):1673–8.

Supporting Information

Data and code availability

Primary data and code for analysis of data have been deposited with GitHub: https://github.com/paytonutzman/Lost-City-MutY-Discovery. These include FASTA files for the discovered MutY enzymes, predicted structures, virtual docking outcomes, MD trajectories and scripts to analyze these trajectories, rifampicin resistance frequency data, and the *R* code to report statistics, median and 95% confidence intervals.

S1 Dataset. Alignment of Lost City MutY homologs

Chemical motifs are highlighted in columns. Alignment was generated by *Promals3D* guided by the structure of *Gs* MutY. It was necessary to align sequences in the first block including up to N146 separately from the second block and third block because otherwise the C-terminal domain residues were aligned inconsistently (see **Materials and methods**). The homologs flagged with dark red highlighting were eliminated because of missing chemical motifs. The homolog flagged with light pink highlighting required manual adjustment so as to align the H-x-FSH motif. The representative LCHF MutYs have the following contig ids: *Marinosulfonomonas* MutY, c_000001803648; *Rhodobacteraceae* MutY, c_000002747260; *Thiotrichaceae* MutY, c_000000598175; *Flavobacteriaceae* MutY, c_000001535696.

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Conservation:                    9 5 579 67999 5  5   65  7         55      6   9 98989 9   5      5       8        65969 67  66 5          859
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sp_P83847_MUTY_GEOS     34  RDPYKVWVSEVMLQQTRVETVIP-YFEQFIDRFPTLEALADADEDEVLKAWEGLGYYSRVRNLHAAVKEVKTRYGGKVPDDPDEFSRLKGVGPTVGAVLSLAYGVPEPAVDGN  146
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c_000002762689_1        31  KKQYYTLVSEFMLQQTQVATVIP-FFNRFIKNIPDLEALANFDNHKLIKLWEGLGYYSRVRNLKKTAQIVVKNFDKKLPRKYLDLKSLPGIGDTTASAISAIAFNKRIIPLDGN  143
c_000005516980_2        31  KREYYTLVSEFMLQQTQVSTVIP-YFKNFIKNIPNLKMLAKVQNKKLIKLWEGLGYYSRARNLKKTSQVIIKNFKGKVPENFEDLKSLPGIGNTTASAILAIAFNKPYIPLDGN  143
c_000003920004_1        37  PNPYYVLVSEFMLQQTTVNTVIS-RFKDFIKKWPNLKKLSMINENQILQFWSGLGYYARAKNLLNSAKIISLKFNNIVPDNYNDLIDLPGVGDTTAKAVLGIGYNKSVMPVDAN  149
c_000004008511_2        31  PDPYFVFVSEFMLQQTTVGTVKT-RFEEFILKWPSINDLARISEKTILNFWSGLGYYSRARNLLKAAKIIKKEFNSKIPNNYDDLINLPGIGDTTKAILGIAYNKSVMPLDVAN  143
c_000004481347_1        38  TNAYYVLVSEFMLQQTTVNTVTK-RFNEFIKLWPSIDKLSRISENRILRFWSGLGYYNRATNLLKTIKIISRDFKFKVPQHYDQLIELPGVGDTTAKAILGIAYNQPVLPLDAN  150
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c_000000754657_2        30  HDLYKIWISEVMLQQTRVNTVVP-YYLRWVEKYESIKSVAKADELKLLKLWEGLGYYSRCKNFHKACKIIVNDYDGEVPSDYQKFLLLPGVGDTTASAVFSIGLKQTYAAIDGN  142
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c_000001286181_5        24  NDPYKVWVSEIMLQQTQVSTVIP-FYENFMKSFPDVLTLSKANLDEVLNNWAGLGFYRRAKNLHQTAKIITDKYHQVFPNNYEDLVALPGIGESTAGAILALAFNKKGTILGDN  136
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c_000001614067_2        37  SNPYFVYLSEIMLQQTVVKTVIP-YFLKFVRKWPDINALAKAELHEINSYWAGLGYYSRAKNLHETAKIISNKYDGFLPTDKNSLMALPGIGETTSSAIMAIAFDRKSNVVDGN  149
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c_000001961666_1        18  VDAYKVWVSEIMLQQTTVNAVIS-RYKKFISRFTNVKVLAKAPLEEVLEEWAGLGYYSRARNLHSSAKIIVNKYEGIIPSEENKLINLPGIGTTSGAIRAIAFNRPSLAIDVN  130
c_000002018097_4        25  NEPYNIWLSEVMLQQTQVKTVVP-YYTKWIKKFPTLKSVAESDLNSLLKLWEGLGYYSRCRNFHKATQIVVEKYKSVVPNDYKLFRQLPGVGDTIASAVLSIAYNKKYPAVDAN  137
c_000002843512_36       26  DDPYHIYLSEVMLQQTQLKTVLERYYFPFLKRFPTLKTLGETPLDDVLKMWEGLGYYNRAKNLHKTATLIEE-----LPSNIDELIKLPGIGKNTAHAVATFAYKKPVPIMEAN  134
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c_000003033795_2        22  RTPYRVWVSEIMLQQTQVNTVIP-YYKKWIKKYPTLKSFKESNFDDVIKIWEGLGYYSRCHNMFNAAKLI----NSTFPNNYDDLINLPGIGDTTAKTILAIAFKNFVGIDTN  130
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c_000004013286_6        44  RDPYAIWVSEIMLQQTQVNTVVP-FFERFIESFPTVEKLAAATEAAVLKRWEGLGYYRRARNLHRAAREVTDQYDGQLPRDPATLSRLPGIGQYTAGAILSIAFDCREPIVEAN  156
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c_000002701031_2         24  SDSYKIWLSEIILQQTRVEQGLP-YFKEFITKFPKIELLAKASEDEVLKLWQGLGYYSRARNMRLTAKAIVEKFNGVFPKDYEKILLKLKGIGPYTAAAICSFAYNLPYAVVDGN  136
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c_000004750284_20        32  VTPYKVWVSEIMLQQTQVATVIP-YFNRFMASYPSVFELANASQDDVLHHWTGLGYYARARNLHKAAKTLVEDYEGVFPPTLDEVVSLPGIGRYTAGAILSLSRDERYPILDGN  144
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c_000004612302_3         35  RDPYAVWIREIMLQQTTVKAVVP-YLERFLDQFPTIEVLAASETDEVLRVWEGLGYYSRARNLHRAARMIVEEWGGQWRETAVELQALPGVGPYTAGAIASFAFDQPAAIVEAN  147
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c_000003136334_1         33  MDPYRVWLSEIMLQQTTTITVRG-YFEDFVSRWPSIEDLAAAELDAVLHAWQGLGYYARARNLHKCAKLITKEYGGKFPEREEALVKLPGIGPYTAAAIAAIAFGHIATPVDGN  145
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c_000001279808_52        28  RSPYYVWISEIMSQQTQIDRVAEKFFPRFIATFPTVEALAAASWDEVYPVWEGLGYYNRGKNLLKAAQIIVEKHDGEIPQTAVELEALPGVGKYTAHAVLAFAFDQKVPAIDTN  141
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c_000003745941_1         22  AALYPTVVSEFMLQQTQVKTVLP-YFHRWLKRFPDFKSLAKAKEETVLKHWEGLGYYSRARNLHRLAREILALN--SIPEDPESWQQFKGIGAYTAAAITSISFNHPVACVDGN  132
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c_000005543774_1          1  KKLYYRILSEFMLQQTQVKTVIP-YFNNFVKKVPSLKALSKSNEKKILKLWEGLGYYSRARNLHKTSKILVKKYDSQIPDDSFEKLKELPGIGDYTANILALIYNHPRIGIDGN  113
c_000006211484_1         23  KKQYYRILSEFMLQQTQVKTVIP-YFNNFVKKIPSLKALSNSDEKTVLKLWEGLGYYARAKNLHKTSKILIKKYNGQIPKSFVKLKELPGIGDYTANILALIYNQPCIALDVN  135
c_000002718976_3         32  KKLYYRLLSEFMLQQTQVKTVIP-YFNKFTKKFKTIKALSKSNEREILKMWEGLGYYRRARNLLACSKILVKNYKSRLPRSIIEIKKLPGIGDYTANALLGLVYNEPRIAVDGN  144
c_000002992548_1         33  KEQYYRILSEFMLQQTQVKTVIP-YFKNFINKIPNLKVLSSSNEKTILKLWEGLGYYSRARNLHKTAKILTKNYKGKLPKEFDEIKKLPGVGEYTANALLALVYNQPRIPFDGN  145
c_000000456751_1          1  ----YVLVSEFMLQQTQVKTVIP-YFTKFVKKIPNLKSLSTSNEKKILKLWEGLGYYRRARNLHKTAKILIKNCKGKLPKKFAEIIKLPGVGEYTANSLLALVHNKPSIPIDGN  109
c_000002655634_1         28  KKEYYVLVSEFMLQQTQVKTVIP-YFKKFVTKIPNLKTLSTSNEKQVLKLWEGLGYYRRVRNLHKTAKILLKQYKAKLPKKFQEIIKLPGVGEYTANSLSALIHNKPCIPIDGN  140
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6u7t.before.V147.pdb     28  RDPYKVWVSEVMLQQTRVETVIP-YFEQFIDRFPTLEALADADEDEVLKAWEGLGYYSRVRNLHAAVKEVKTRYGGKVPDDPDEFSRLKGVGPYTVGAVLSLAYGVPEPAVDGN  140
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sp_P17802_MUTY_ECOL     141  VKRVLARCYAVSGWPG------KKEVENKLWSL----SEQ-VTP---------AVGVERFNQAMMDLGAMICTRSKPKCS----LCPLQNGCIAAANNSWALYPGKKPK-----  225
sp_P83847_MUTY_GEOS     147  VMRVLSRLFLVTDDIA------KPSTRKRFEQI----VRE-IMA---------YENPGAFNEALIELGALVCTPRRPSCL----LCPVQAYCQAFAEGVAEELPVKMKK-----  231
c_000003652391_2        150  VERVMARLFDISTPLP--------GAKPALKAR----AAS-LTP---------KLRPGDYAQAVMDLGATICTARAPTCG----VCPLNDCCHARRAGTAAELPKRTPK-----  232
c_000000031207_6        139  VERVLSRYLKIDGNPK------SGTARKRIQQA----ADD-ALD---------QENPGDHNQALIDLGARSICTPRSPDCQ----RCPLASGCGARASGEPTRWPTRRQK-----  223
c_000000430975_4        151  VERVVSRIFQITQPIP--------LSRPTLRQL----AAG-LTP---------SKRPGDYAQAIMDLGSLVCTPRKPKCN----VCPLHDICLANISGTPSDFPKRTPR-----  233
c_000001007148_4        148  TARFYARLLALRSDLH------SGEGRRQLWSL----AER-IVP---------RDRPGEFNQALIDLGATVCGV-TPRCQ----ECPVRGSCRSLELGEVERIPARASR-----  231
c_000001092848_3        161  VERVITRLRAIGTPLP--------AAKPQVRKA----VAA-LVP---------AERPGDFAQAMMDLGATICTPRRPSCI----LCPLQAFCAARGAAAQESFPVKASK-----  243
c_000001151029_1        108  VERVVSRIFQITQPIP--------LSRPTLRQL----AAG-LTP---------SKRPGDYAQAMMDLGSLVCTPRNPKCN----VCPWHDICLANISGTPSDFPKRTPR-----  190
c_000001797282_1        170  VERVLSRAYAVEAPLP--------GSRPEIRRL----TQA-LVP---------PDRPGDFAQALMDLGATICTPKRPACA----LCPWMRPCRARSLGTQESFPRKIKV-----  252
c_000001803648_25       153  IERVMARMHDIHSPLP--------SAKPELLAC----AAA-LTP---------DFRAGDYAQAVMDLGATICTPKSASCG----VCPWSFACLARINGTAAVLPKKTPK-----  235
c_000002078955_4        153  VERVMARLHNIHTPLP--------AAKPELFSK----ATA-LTP---------DLRAGDYAQAVMDLGATICTPKSPTCG----ICPWSCACVALISGTAAELPKKTPK-----  235
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c_000003607531_2        151  VERVITRVFGIFKPLP-----EARPIIKDCAAK----LAP-KTT---------RGRPGDYAQAVMDLGATVCRPRKPLCD----LCPWEKYCIANQQNFVDQVPRKAPK-----  236
c_000003872363_4        149  VERVMARLFNVITPLP--------AAKPELTAH----AAA-LTP---------VDRAGDYAQAVMDLGATICTPKSPGCG----ICPCTEYCAALPEGDAANLPLRPPK-----  231
c_000004820107_1        156  IERIMARLYAITEPLP------DSKANLKNLAA----GLS-EER---------KDRPGDYAQALMDLGATICTPTSPKCS----LCPVNDSCTAKRQGIAESLPIKKKK-----  240
c_000005774797_1        140  INRVLSRVYNIDDAIN--------RPSARLARI----AET-LAH---------PKRSGDVAQAMMDLGAAICKPKNPNCE----TCPICDQCKAHSLGRTHELPVKAPQ-----  222
c_000005867021_3        153  VERVMARLYNIHSPLP--------AVKPMLLTH----ATA-LTP---------EFRAGDYAQAVMDLGATVCTPKSPTCG----ICPWSFDCIAHAKGTAAQLPKKTPK-----  235
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c_000004551008_5        144  VKRVLSRFFGVKGWSG------ESKVSKELWDL----SAK-SLP---------VDNFEIYTQGIMDLGATVCLPKNPNCS----NCPIVNKCYSYLNQLVDEIPSKKLA-----  228
c_000002511148_4        139  VKRVLARHFKIEGKLE------TSSKRKELWQI----SED-LLP---------DNKIEVYTQAIMDLGATLCIKSSPACN----LCPLNQDCLALKEDLVTVLPSKTKP-----  223
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c_000000598175_2        150  VKRVLTRVFVVDGWPG------KADVLKKLWLL----AEQ-TTP---------ASRNADYTQAIMDLGATLCTRSKPRCE----DCPLKADCLAFQQGNQTVYPTSKPK-----  234
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c_000000899687_2        142  VKRVLSRLFCINKNGT------TSAFENCLWGE----AED-LLP---------VRRPGDFNQALMELGATVCIPASPICQ----QCPLRTICKAFLKNKVNEFPPSKNK-----  226
c_000001176522_24       161  VKRVLCRLFGVEGWPG------ERQVEAHLWTL----AEC-LLPA-------QSEVMPDYTQAQMDLGATLCTRTKPRCE----ACPFQAECVAYRQGRVAELPAPRPR-----  247
c_000001345122_1        117  VKRVLCRFDAVTSWSG------NKKTEKVLWDR----ADE-FTP---------QERFDDYTQAIMDLGATLCTRSKPKCD----VCPVEADCDAKKMDKVIQFPYSNPK-----  201
c_000001515736_6        131  VKRVLTRVLAFDGDLA------QSRNERQLWEY----AQQ-LCPTE-----NLHEAMPRYTQGMMDLGASVCTSRKPTCL----VCPLDSECRAARAGNPENYPVRTRR-----  219
c_000001682161_6        138  VKRVLCRFDAINSWSG------NKQTESQLWQR----AEQ-LTP---------SKRVKDYTQAIMDLGATVCTRSKPSCE----ICPVAENCQARQLNQVAKYPFSKPK-----  222
c_000001923643_29       141  VKRVLTRVLAFDADLA------VARNERELWDL----AQQ-LCPTE-----DLQQAMPRYTQGLMDLGATICTPRKPSCL----VCPLQPQCRAARFGNPENYPVRTRK-----  229
c_000002030994_5        139  VKRVLSRVFTVVGWPG------KADVLKKFWVL----AEQ-TTP---------KTRNADYTQAIMDLGATLCTRSKPRCE----DCPLKTDCLAFQQGNQTEYPTKKPK-----  223
c_000002038721_4        141  VKRVLLRVLGNKSPIN------HSKTQKELKKF----AAE-LLP---------KNDFRTYSQGIMDLGSMICTPINPTCK----NCPIQNKCKSFQLNLQNMIPVKTSK-----  225
c_000002523527_15       156  VKRVFARVFGIHGYPG------ERAIENRMWAL----AEQALPPAG----RHQADHMVAYTQGLMDLGATICSRGKPACLADAEACPLVADCVARKDNLTAVLPTPKPR-----  250
c_000002608528_2        139  VKRVLTRVFTVEGWPG------KSGILKKLWLL----AEQ-TTP---------QTRNADYTQAIMDLGATLCTRSKPRCE----DCPLRAGCLAFQQGNQTDYPTKKPK-----  223
c_000002961510_2        142  VKRVLSRLFCINENGT------TSASENRLWQQ----AED-LLP---------VRRPGDFNQALMELGATVCIPKSPICQ----QCPMRTLCKAFLKNKITEFPPSKKK-----  226
c_000002979152_8        147  VKRVLARYHAIEGWPG------KSAVLKELWEK----AEL-HLP---------AERNADYTQAMMDLGATLCSRSKPACE----RCPVVDGCAAARKSSWAYPGKKPR-----  231
c_000003248034_1        159  VLRVLTRVLDEKGNVA------STAIQQKLTAA----ARL-LVES------VGPHSAGTWNQALMEVGATVCTPQSPSCD----RCPINRWCSAWKAGTQKTRPVKARP-----  246
c_000003294679_3        144  VRRVFTRHKAIKGWPG------TTMVQNVLWKI----AED-NTP---------SNRVADYTQAIMDLGATICTRSHPRCE----LCPVNKDCIAHSRGQEAEFPHKNIK-----  228
c_000003333364_1        141  VERVLARLFDVDAPIK------SSQAQSFLWET----ARS-LIP---------TGQAREFNQAIMEFGNLVCGR-KPSCM----FCPLTEECEAHYLGIVEHRPVPAER-----  224
c_000003402697_5        142  ISKIITVLWPG----------------QDTVEV----ANE-LVS--------LSQSGYAWNSAMMDLASSLRAGNPVE------GALGETYFSDEAICRLFKPKRKKPEKKIK  219
c_000003492925_4        134  VKRILCRLHKLKTP----------SDKELWRI----AYN-LVD---------KKNFDYNQAMMDLGATLCTPKKPDCE----TCPLADICQGQNDPTLYPTKKKR-------  211
c_000003766664_5        154  VIRVLSRLCDLEEEVG------ETKAKERIWDW----AER-LVD---------PQNASAFNQGMMELGAMLCTPTSPGCD----QCPWREDCRARAAGTVLERPIKKAK-----  238
c_000003948186_72       141  VKRVLSRVHRVAGWAG------RASVNKELWQL----AER-YTP---------KRRVERYTQAIMDLGATVCRVGQPRCH----ECPVAKHCEALRHDCVQSFPQPKAR-----  225
c_000005057120_4        140  VKRVLTRYFAISGWTG------NSKISNELWEI----SSR-YTP---------KIRVADYTQAMMDLGATLCTRSKPQCT----ICPIQADCLAKKQNKTAELPTPKPA-----  224
c_000005590109_5        140  VRRVLARFHVISGWTG------GTKVSNKLWQL----SSD-YTP---------LLRIADYTQAIMDLGATICIRSKPKCT----QCPINSACLAKIEGKISELPTPKPK-----  224
c_000006063368_2        139  VKRVLTRHRKIETDLS------KSSTLKKLWKL----SEL-LLP---------DVRIDTYTQAIMDLGAIVCKKKSPLCE----ICPVNKDCLALKANLVDCLPVKRKI-----  223
c_000000467631_2        119  VKRVLARYHAISGWTG------SAKISSELWKI----SAR-YTP---------AERCADYTQAMMDLGATLCTRSKPRCG----ECPISSDCVARIGDKVKLLPTPKPA-----  203
c_000000788235_1        140  VKRVLTRYHAIEGWSG------KGHILKQLWSI----AES-HLP---------DTRYANYTQAMMDLGATVCTRSKPKCL----GCPIHADCSAFQAGTPTAYPTPKPK-----  224
c_000001187176_11       141  VKRVLARYHAVQGWPG------EKPVHDRLWAI----AEA-HTP---------HSRVAHYTQAMMDLGATLCTRSKPSCL----ICPLQAGCQARMLGEPTAYQARPR-----  225
c_000001660697_64       141  VKRVFARVFGIDGFPG------DKRVEDTLWRI----AEAVLPP---------ADGIQPYTQGLMDMGATVCTRGKPACLTGERACPLESLCEARSTDRVMELPVPRPR-----  230
c_000003378864_2        152  VKRVLSRLYDVSDP----------VGTAAATRTLWAYSTELLN--------QADDAYTHNQAIMELGATLCTPKKTACT----RCPVSVRCDALARDTVPLRPIKRPR-----  237
c_000003910742_3        141  VKRVLTRYHAVEGWTG------KSQVLKQLWLL----AES-HLP---------ATRYANYTQAMMDLGATVCSRTKPDCD----HCPLNSNCAALSKGNPTAYPTPKPK-----  225
c_000004116181_2        107  VKRVLARFNAVQGWPG------NASVNKQLWAI----SAR-YTP---------VERVADYTQAMMDLGATVCTRTRPGCQ----ICPVNENCLARKKSLTEIIPASRPV-----  191
c_000006223903_1        142  VKRVLSRYHAVEGWSG------RAEVARRLWSL----AEA-HTP---------TRRVAAYTQAIMDLGAMVC-RRRPDCA----TCPLAGGCKAHKQNQVHDYPASKPT-----  225
c_000001029068_2        114  VKRVLARYHQVDGHYG------QGKTIKQLWKL----SEK-HTP---------NTRNNDYTQAIMDLGSIVCTRLNPVCD----CCPVALRCLAKKTHTQHLYPASKPK-----  198
c_000001128125_11       149  VKRVLSRYFGIHGWPG------EKKIEADLWHK----ADL-LTP---------PKRFADYTQAIMDLGATTCKRSKPLCG----VCPVQKSCVALKREETHSIPASKPK-----  233
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c_000001595844_4        140  VFRVLARYTANFIDIG------TPAGKKEFTLL----ANQ-LLD---------KKQPGKFNQAIMDLGALVCKPTNPDCS----NCPLQIKCQAFSQDTIAQLPVKEKK-----  224
c_000001719155_2        144  VKRVLSRLFLLKENGE------TRKSENILWET----MQQ-LLP---------ETGAGNFNQAIMELGATVCLPKNPLCL----LCPLKRNCQAQKSGKQNLYPPRKRA-----  228
c_000002363038_6        149  VKRVLSRYFAIEGWPG------EKRVENQMWGL----ADN-LTP---------ETDFADYTQAIMDLGATLCKRSKPNCN----LCPVKTDCRALKTDQVASFPNSKPK-----  233
c_000002561400_1        105  VKRVLTRFFGVHGWPG------EKQVENRLWHR----ADE-LTP---------SVRFADYTQAIMDLGATLCKRSKPNCI----TCPVHSGCQALKKGEVHLLPSFKPK-----  189
c_000002566035_2        141  VKRVLTRYHGIKGWPG------EKKIENKLWSL----AEL-YTP---------RKLTKQYTQAIMDLGATVCTRRKPDCD----ICPLTLTCFAREMGKQHDFPGKKPT-----  225
c_000002598045_3        139  VKRVLARHFKIEGNLQ------ASSTTKQLWQI----SES-LLP---------GDRIDIYTQAIMDLGATVCTKSNPLCS----NCPVNQDCSAFKEGLVDTLPLKVKR-----  223
c_000004188575_41       141  VKRVLARFYAIASWPG------NKATEQQMWQQ----ADN-LLP---------DQRIAAYIQAQMDLGATLCTRSKPNCD----CCPLQSNCQAYARGTPTDFPIAKPK-----  225
c_000000240049_1        139  VKRVLARHQAIEGWSG------QSPVSKKLWTI----AEQ-FTP---------ENQLADYTQAIMDLGATVCTRSSAKCG----VCPLSEDCLAYQQQRVADFPGKKPK-----  223
c_000002139456_2        139  VKRVLARYQAVEGWSG------QSQVSKKLWAI----AEQ-LTP---------KEQLADYTQAIMDLGATVCTRSTAKCG----GCPLSEDCLAHQQQTVADFPGKKPK-----  223
c_000003535614_2        141  VKRVLARYQAIEGWPG------KPAIEKQLWVF----AER-YTP---------TEELADYTQAIMDLGATLCTRASPKCG----SCPLNNGCKALANNCVAALPTRKPK-----  225
c_000002657784_4        138  VKRVLARYKKIEGDLS------KALNIKTLWRI----SES-LTP---------EEKVDLYNQAIMDLGALVCTKSSPKCR----ICPVSRDCIAFNKKLIQVLPRKKLT-----  222
c_000003800129_15       143  VKRVVGRLYNQESY----------QEKKFWEL----SKK-ILD---------KSNPFLFQQGIMDIGATICHIHNPQCD----ECPLGRWCLSKKNGSFYTYKKKK-------  220
c_000003716781_1        130  VRRVLARWFAVEGVV------TKAEPRREIEGL----AAA-LVG--------GVNDPGSWNQALMELGATVCLPKDPRCD----SCPVMTECRARARGLERAVPELPVR-----  215
c_000001556689_3        131  VARIMCRLAGIADDTR------RSKVRQRLEAM----AAD-AIE---------CHPPGTFNQALMELGARVCTPRSPRCD----QCPCSSYCEAHELGIEEQIPPRRSK-----  215
c_000001834452_1        108  VCRVAARFLGLKSPLG------SSALRGQARDW----GET-LHAG------VSAGLAGSLNEGLMELGATVCRPRAPLCG----TCPISEKCVARATNRVTEIPKKAKR-----  195
c_000004255004_2        148  ARRVYARLLSLTN----------LRAINET----AEQ-MVS---------HSRPGDFNQAVMELGATVCLPGKPRCE----RCPLAYRCQARSVGAFKLQLRRPTR-----  225
c_000000605438_3        141  VKRILYRFFAVKEA----------NDKKLWEM----AYD-LYD---------KDNAYIYNQTMMDIGSAICTHKNPLCT----QCPFVSLCQGKDEPL--LYPTKKKK-----  218
c_000000581237_15       141  VKRILYRFFAMTSC----------NDKKLWEM----SYE-LYD---------RENSYIYNQTMMDIGSAICTHKNPACD----VCPFEPLCQGKESPL--LYPEKKVK-----  218
c_000002529579_2        141  VKRILYRFFAVKEA----------NEKKLWEM----AYD-LYD---------KENAYIYNQTMMDIGSLICTHKNPLCT----QCPFVSLCQGKDEPL--LYPTKKKK-----  218
c_000006097838_1        114  VARVLTRLLGIERPSK------QKQVVEQLWSV----TAD-LVKHAVDLQKTSRRNATAFNQAMMELGALICTPRNPLCH----DCPLKGHCIARKQNKVNRIPVSAKR-----  207
c_000000583727_6        140  VARVLTRLLGIEQSPK------DKPVTDRLWSV----ADE-LVKHTVTLQKPRQRNASAFNQAMMELGALVCTPRNPLCG----DCPLKGRCTARKQNKVETIPALAKR-----  233
c_000005807640_2        142  VARVLTRLLGIEESPK------ARPVADRLWSV----ADE-LVNHAVTLRKPSQQNASAFNQAMMELGALVCTPRNPLCG----DCPLKGRCIARKQNKVETIPALAKR-----  235
c_000000134878_1        124  VNRVVSRFYAYQDPPS--------KNKKKMEKF----MSL-IIN---------DNRPGDINQSVMDMGRYICKPSTPLCD----ECPIHNGCKALKLGIQSDLPIKIKK-----  206
c_000005136725_3        139  VNRVVSRFYGYRDPPL--------KNKKKIEKF----MNA-IIN---------DKRPGDINQSVMDMGRYICKPRTPLCD----RCPIQYGCKALKLGIQADLPVKIKK-----  221
c_000001684786_4        137  VERVMTRILRLAENPR------QVSTKKRLKEI----AGG-WMP---------ADKASSFNQAMMELGALICSPVSPDCP----SCPVREVCAAAEQGDPENFPRLPPK-----  221
c_000002971826_23       147  VERVLTRILRLADNPR------TAATKKYLKEI----AGG-WMP---------RGKASSFNQAMMELGALICVPVSPNCA----ACPIHDACAAAEHGDPENFPKLPPR-----  231
c_000002040695_2        150  IARVFSRVLQYPGSKE--------ALTTSLRQP----ISL-FLP---------FKRIGDYTQALMDLGALLCRPCNPQCS----LCPIKTLCKSAHHNTVHLFPHKTHK-----  232
c_000003263657_32       154  VIRVLSRLYRIHEDAT------KGPAKRRFQEL----ADG-LLQ---------KGQAADFNQAMMELGATVCLPQKPRCT----VCPVQCHCRAFHELSDPSGLPYKPP-----  238
c_000005989041_2        151  IERVITRIFGICTPFP-----EAKTIVKDCAAK----LAP-KTT---------RGRPGDYAQAVMDLGAIVCRPKKPLCD----LCPWKKCCIANQRNFVDQVPQKVSK-----  236
c_000002391082_2        149  VTRVLCRLLRIEEDPR------RTAIEAELIAA----GEA-LLA---------RGEAGDFNQAMMELGARVCTPRNPLCP----TCPVGQWCRAQAELDDPSALPYKVP-----  233
c_000003839553_2        156  VIRVLCRHLRIEGDPR------RAQIKAELIAA----VEA-LLA---------RGQAGDFNQAMMELGARICTPRKPLCE----TCPVGATCRARAELDDPTALPYKAP-----  240
c_000003996707_2        146  VTRVLCRLLRLEGDPR------KAAVKTELIAA----GER-LLA---------RGRAGDFNQAMMELGARICKPTSPACG----QCAVVEMCRAHAELDDPAQLPTKVR-----  230
c_000002044706_1        129  VIRVLTRLFRIPGDPG------TAAVSRRLWQL----ATD-VLP---------QNRPGDFNQALMDLGATVCKPIAPLCE----QCPLAGLCAVRAAGNAEDFPNAGRR-----  213
c_000006007075_1        144  VERIIKRILNLKTEKE--------ISKENIIKK----KKI-LGM---------SDRSSDYAQALMELGALVCKPKNPYCK----KCPITKNCLSYKKKDFEI---ISKN-----  223
c_000002094036_4        120  IERVLKRILNLKSEVK--------TKKENLHKQ----KKV-FGQ---------TNRSNDYVQALMEIGALICKPKIPHCN----KCPITKNCLSYKKKDFKI---KPKN-----  199
c_000002762689_1        144  IERVLKRYLYLKKENE--------INKENLIKK----KKI-FGY---------SNRASDYAQALMELGALICKPNNPHCE----QCPISNKCIALKKKDFLL---TKIK-----  223
c_000005516980_2        144  IERVLKRYLYLKKENE--------IQKENLIEK----KSV-FGL---------SQRSSDYAQALMELGALICKPSNPLCN----QCPISKKCKSFRKKDFNL---IKNT-----  223
c_000003920004_1        150  IKRMLARLYGLDQSIN--------LINKKITSL----SKF-YES---------KKQSSNLIQAFMDYGSIICVPRNPKCG----ICIISRECIANQRKISNIIPKKIKS-----  232
c_000004008511_2        144  IERILVRLYGLKLPII--------KIKNDLRKK----SDN-FIS---------KNSSSDLIQAFMDFGSLICTPRNPDCV----NCLIQKNCNAFNYNNLQDIIPVKLKS-----  226
c_000004481347_1        151  IKRIIARLYGIKTSLL--------LNKKTIEDI----ASK-YQS---------SNKASDLIQSFMDYGSVICLPRNPKCD----ECLIEKFCEARKKNIQHLIPFKNLS-----  233
c_000003787733_3        143  VKRVMARILKIKHITK--------RNNRRIRNR----LIK-WMD---------PERPGDINQALMDLANKICRVDHAYCN----TCPIDKVCMAEKMSIPESYPTKLNK-----  225
c_000000754657_2        143  VRRVMSRLLRIKNITK--------RNKKRIHNT----LIE-WMD---------RERPGDINQALMDLANKVCRVDHAQCN----SCPIDEICMANKMSIPESYPTRIKR-----  225
c_000000990943_3        143  VRRVMARVLRIKNITN--------RNKKRINNT----LIK-WMD---------PVRPGDINQALMDLANSICRVDHAHCS----ICPIDEICMAEKMPIPESYPIKVKK-----  225
c_000004474996_2        142  VIRVMARLLRIKNITK--------RNKNRIYNT----LIK-WMD---------KERPGDINQALMDLANKICCVDHALCS----TCPIDEICMANNMSIPESYPAKLEK-----  224
c_000001463500_11       130  VTRVLSRILAIRNLTP--------YNRRRIFNV----LEK-FID---------KERPGDVNQALMDLGSSICTPINTECI----ICPVSQFCKAKKTLLPFGYPIKINK-----  212
c_000001286181_5        137  VKRVVSRYVNVENNPR--------ELKKSIKPF----LYQ-NSP---------KANYQSFGQGMMDLGSLICVKEKPKCD----LCPIQKTCLSFKKQDFIKTKKNT-------  217
c_000001293628_3        143  VRRVLSRLFDLADP----------TDASLRTR----AGE-LVD---------PDRPGDWNQALMELGATVCTARSPRCS----VCPLTKQCRAETEGTQELRPAPKRR-----  222
c_000001535696_8        137  VYRVLSRYFGVDLPIN------TTEGVKYYKEL----AFK-VMD---------SQNIRDYNQGIMEFGAIQCAPKNPNCF----SCPLSDSCVALQENKIDTLPVKLKK-----  221
c_000001614067_2        150  VERVFSRFYAVEKPIK--------ESKIFIKNI----AEK-HLP---------DNRHGDYAQALMDLGSLICIPKSPRCN----MCPLLAICDVGGTTSAKQYPIKLPK-----  232
c_000001765289_1        116  AKRVMSRILGIKNL--------TSWNLSRINKT----LSN-IIP---------EHTPGNFNQSVMELGATLCRPRSPSCN----KCPLSFGCKA-FKTNKPDYYPKPAA-----  197
c_000001961666_1        131  VERVITRLFKIDNNK---------KVKNKIKKY----TQS-LIP---------QNKPGDFAEALMDLGSLICKRANPTCS----LCPLKLNCKGYISNQIKVSVKNKKL-----  212
c_000002018097_4        138  LKRVISRYLGIKKLTR--------RNTLRIHNQ----LNK-MLT---------NGRPGDINQALMDVGSLICKPNDVICI----KCPLINKCKGYSYGNPIMYPDKNKR-----  220
c_000002843512_36       135  VRKILCRLQKLKTP----------TDKELWKI----AYD-LVN---------KKNPFDYNQAMMDIGATLCTPKNPNCE----ACPLSDICQGQSDPALYPTKKKR-------  212
c_000002930199_1        135  VYRLISRLYNINTAIN------TNKGREEFQSI----ANN-LLP---------NKNTGLYNQAIMDFGSIQCKKYNPKCN----ICPLQKECQSAILGIVNERPVKTLS-----  219
c_000003033795_2        131  LERIGYRILGLKTKTK--------RNKKRVVEY----LEK-NQC---------ANNPGDYNEALMDLGSSLCKASITYCN----QCPLKKICKAYTSSSPILYPTPKVP-----  213
c_000003888107_2        132  LERIGFRLLGLKKRSK--------FNQNKVKKF----LEK-IQD---------KNNPGDFNQALMDLGSNICQSQSVNCR----VCPINDSCRAFLSLNPVDYPAPIKP-----  214
c_000004013286_6        157  SRRVLSRVFAIKGDLS------RQPMKHLLWDL----AKF-VLP---------ENKVGDFNQAMMELGSQCCQKQYPRCM----QCPIKEWCRAFQEGLQEELPFTPIQ-----  241
c_000004852258_1        119  HSRIIARVLGVKNQTS--------RNLNRINNY----LKK-LVR---------EGNPGEINQALMDMGSNICKSNKVICV----NCPFHFSCKAFQSGHPLSYPKKILP-----  201
c_000005254087_1        145  VVRVLSRLTDFAEDVT------TTPAKKQLWQM----AAK-LVP---------ADRPGDYNEAIMELGQTLCLPTKTDCV----ACPLTAVCLARQRGTQMERPVRPPR-----  229
c_000005603677_1        142  VYRVLSRYFGQALPID------SSAGKKWFKAK----AQA-LLW---------EDDPANYQAMMDFGALHCRPKNPSCG----DCPLAKNCQAKANKQQLELPKKGKK-----  226
c_000003283462_3        130  IKRIMARMLGVKKITN--------FNYKRIKKT----LLN-LIS---------SSRPGDFNQALMDLGSQVCRPNQAHCY----RCPLKLFCKAGNTLNPEKYPRPLSK-----  212
c_000002687221_1        144  AIRVISRMNSIDSPYP--------KSRKKIINF----LSE-HID---------TIRPGYFNQALMDLGRVICTYKSPSCY----ICPVNTYCYSYVNNTVDKFPTKIKK-----  226
c_000003347358_19       166  VKRILCRLEKLRTP----------SDTELWDI----AYT-LVD---------KINPFDYNQAMMDIGATVCIPKKPQCD----ICPLNDICKGQEEPTLYPTKKRR-------  243
c_000002701031_2        137  VIRVLSRYFGIQTPFD------SSDGKKEFQLL----AEE-LLI---------KEKAGENNQAIMEFGALQCTPKSPNCN----VCPLQNTCFAFNNNLILELPKKAKK-----  221
c_000000582753_3        145  VYRFISRLYGVSTPIN------SGKANKEFKVI----LNA-IID---------EKNPATFNQAIMEFGSLQCTPKSPNCE----SCTFSEICFAYKNNVIKDLPVKLKA-----  229
c_000001713769_5        137  VYRVIARFYGIDVPVN------NHLGKKFYMDY----AQK-LAP---------KKSCGDYNQGIMDFGSLICKPKAPLCD----KCMLAKDCIASKMKNVNYFPVKLKK-----  221
c_000004369364_1        140  VFRVLSRYTANFSDIG------TPVGKKEFTLL----ANQ-FLD---------KKQPGRFNQALMDLGALVCKPVKPICP----DCPLHSDCLAFAQDIISSLPVKAKK-----  224
c_000006057486_30       141  VYRVLSRYFGVSTPIN------SAKGIKEFKQL----AQI-LIN---------PKNPGTHNQSIMEFGALMCKPQKPNCT----NCPLNDSCFALQNNQIKTLPIKTNK-----  225
c_000005494072_10       137  VYRVLSRYFGIEYAIN------SSKGIKKFKTL----AQS-LLL---------NENFGLHNQAIMDFGATICTPKKAKCD----SCIFCESCSALQHNKVSELPVKTPK-----  221
c_000001742634_3        137  VYRVLARYFGIKTAIN------STNGIKEFKLL----AQQ-LLP---------NNKIGAYNQAIMDFGATICTPKKPKCD----ICIFNDSCIALQKNKITELPTKTNK-----  224
c_000002826998_2        140  VFRVLSRYFGIETPMD------TSEGKKQFTHL----ANE-LLL---------KKKPAIYNQAIMDFGAIQCTPKSPDCK----HCPLKKKCQALQENKISKLPVKSKK-----  224
c_000003159439_6        137  VYRVLARYFGIRTSTN------STKGIKEFKQL----AQE-LID---------TKDPATFNQAIMEFGAIQCKPKNPNCN----NCPLNTSCIALQKKLITILPIKDKK-----  221
c_000004887214_1        142  VYRVLARYFGIRTSIN------TSKAKKEFKEV----AFE-LID---------SKNPGLFNQAIMEFGSLQCTPKNPTCS----ICPLNDTCVALQTNQVEVLPKKQPK-----  226
c_000005037037_2        140  VIRVLSRFFGIENTFQ------TVKEKKEFQEI----ADV-LIS---------KKSAADYNQAIMDFGAQICTPKNYQCE----KCPISANCFAYKNKQQNNLPLKKKK-----  224
c_000001059964_1        132  MIRVISRMMNNQEEPR------LAKTRKRLWQL----ARE-WIP---------EGEARRFNQAMMEIGALICLPSNPKCL----LCPMNNHCRALQKGTVSLIPLKPPK-----  216
c_000002498472_1        141  VERVFARIFNIDLIPG------SPEARQLNWEK----AEE-LLP---------KGHARNFNQALMELGALICKPKKPLCT----SCPLVTHCLALKYDLVPERPVPKKS-----  225
c_000004615912_3        130  VYRVLSRFFGIKAAID------ISKNKKIFEVL----AQE-VLD---------ASQAGKYNQAIMDFGATMCKPKSPDCP----LCPLSKKCIALKEELVTTLPIKSKK-----  214
c_000004766858_2        143  IERVLCRIEDIGEPPK------STPVQKQLWSL----AAE-WIP---------HGHAREFNQGLMELGATLCTPRNPSCL----LCPANAWCVAFHTGRTDTIPAPRVR-----  227
c_000002747260_18       149  VERVMARLFQIETPLP--------GAKPELTEL----AGR-LTP---------DIRPGDHAQAVMDLGATICTPKSPACG----ICPVMTGCTARAAGAQADLPRKTPK-----  231
c_000005371561_38       149  VERVMARLFDIHEPLP--------AAKPVLKAQ----AAA-LTP---------ATRPGDHAQAVMDLGATICTPRNPACG----ICPLRAPCAARAAGTQADLPRKTPK-----  231
c_000000169465_2        145  VKRVLARYYSIGGWPG------QKKVENQLWEV----AEK-NTPTN-----SEGGRCANYTQVMMDLGAMICTRSKPKCD----ECPLQADCIAYAQGAQADYPGKKPK-----  233
c_000003254110_11       145  VKRVLARYYAISGWPG------QKKVENQLWEV----AEK-NTPTN-----PEGGRCANYTQVMMDLGAMICTRSKPKCD----ECPLQPDCIAYAQGAQTDYPGKKPK-----  233
c_000004750284_20       145  VKRVLARYYAIEGWPG------QKAVENALWDV----AEK-NTP---------QNRCANYTQVMMDLGAMVCTRSKPKCD----ECPLQRDCVAFAQGKQTDYPGKKPK-----  229
c_000000141782_15       150  VKRVLARYHAVAGWPG------ETAVSRRLWAL----AER-YTP---------DNRTADYNQAMMDLGATVCVRRRPRCG----VCPLADGCRARREGNPEAYPGSRPR-----  234
c_000002717847_8        144  VKRVLARLFLQQEF----------KEGLFWNL----SAQ-CLD---------EDDPYSYQQGIMDIGATLCTPKAPLCP----ECPLNKNCLAFKENDFQLPSSKKPI-----  223
c_000004054799_1        131  VKRVLGRYKKISFKT-------ENEKQKKLWLI----SEE-LTP---------NKESFEYTQGIMDLGATHCSKSRPRCD----LCPVSNDCDSAFKEVSNKVSLRKAI-----  214
c_000006126673_1        156  IERVIARYHAINTPLP------KAKKEIKERAT----IYT-DNL---------PERPGDYAQALMDLGATICRPKSPLCY----SCPLKEGCTAYSLGQAEEYPKRQKK-----  240
c_000000339186_3        113  VIRVLTRIFGIRKNPK------DKATREYLWSL----AET-LVA--------KAENSSALNQSLMELGATVCTQRRPDCA----ACPVARSCTARRERATEIIPAKGEP-----  198
c_000003029168_2        145  VARVVSRLFALEGDLK------DTALQKKLWEI----TED-LVP---------NKRPGDFNQAMMELGAIICSTDSPRCT----LCPLAKPCHARVTGEPWRFPSESVT-----  229
c_000001863436_1        161  VARVLARLFAVDGDPR------SGDNQRRFWRL----AGA-LID---------RGRPGDFNQSLMELGATVCTPQGPSCL----LCPVREYCQAFATGDPTRYPAVAKR-----  245
c_000002830137_4        154  TTRVLSRLLAFHGDPS------QRVGQQLLWAF----AES-LLP---------RKRVGEFNQSLMELGSEICIPRKPNCE----RCPVRMLCAAHRQGEQLSIPISRRR-----  238
c_000004612302_3        148  TARLFSRLLALSVDPS------SSEGRGRLWSL----AER-VVP---------RTRAGEFNQALIDLGATVCGV-EPRCQ----ECPVRSSCRSLEQGVVDRVPVRSPR-----  231
c_000001933926_1        134  IERVMSRLHRLETPLP--------AAKPEIAEV----AQR-LTP---------RQRAGDFAQALMDLGATVCRPRRPNCG----ACPWNASCLAHAAGIQETLPRKLPK-----  216
c_000003136334_1        146  VERVTARLHRISKPLP--------GAKKYLKEK----AAE-LTP---------ASQAGKYNQAIMDFGATMCKPKSPDCP----LCPWSYICKAYLAGDAEKFPKRVPK-----  228
c_000005849454_9        143  VKRVAARWAGLELAADDLALEKAARALGEAWMT----DLP-LGK---------LSAPGELNEGLMELGATVCSPRNADCG----ACPLASGCVALADGMVDVLPLPKRA-----  233
c_000001169194_1        154  VRRVLARLFDKREP----------RARWLNRT----AAE-LVD---------KDRPGDWNQALMELGAMICSPKNPRCE----DCPVQLWCTAHEQGTQALSPAGVRR-----  233
c_000002786947_2        154  VRRVLARLFDKHEP----------GVRWLNHT----AAS-LVD---------KDRPGDWNQALMELGAMICAPKNPRCE----DCPVQRWCTAHEQGTQALSPAGIRR-----  233
c_000001279808_52       142  ISKIITVLWPN----------KGTVKV----AES-LVA--------AANSGYIWNSAMMDLASSLRAGNEIE------AELGSTYFADVEVRDQFKPKRKKPSRTAK  219
c_000000358065_2        144  IERVLKRILNLKTEKE--------TKKENLHKL----KKV-FGI---------TNRSCDYVQALMEIGALLCKPRNPYCE----NCPITKNCLSFKKKDFEI---KKKN-----  223
c_000003745941_1        133  VIRILCRLTADNTVYT-----DNTQAMKVFTPL----AEV-LLD---------TKTPGQHNEAMMEFGAMVCSKVNPTCD----ICPFNSVCLGRNHQPER-LPIKAAK-----  217
c_000004187032_4        144  IERVLKRYLHLKKDNE--------IQKNNLIKK----KSI-FGI---------SSRASDYAQALMELGAMICKPKNPECS----ACPILKSCKSYKKKDFDL---AKIR-----  223
c_000006067315_3        144  VERVLKRVFYLKNEKE--------ISKENLINK----KFF-FGK---------SNRSSDYAQAIMEIGALICKPANPLCF----ECPISKNCRSFKKNDFEI---TSKN-----  223
c_000005543774_1        114  VKRVLLRLFNLETKD----------------------PAN-LFK---------TKRNGDLAEALMEFGAIICKPKDPLCY----KCLLKKNCLYYKSESKI-RFNKKIK-----  185
c_000006211484_1        136  VKRVLFRLDNININD----------------------AVN-LFK---------TQRNGDLAEALMEFGALVCRPKDPRCH----ECKIKKMCAYFKSKSKI-KFNRKIK-----  207
c_000002718976_3        145  VRRVFSRNLNIEENK-------INFDKFIKKN----KKK-FFI---------TKRNDDFVEALMEFGALICKPKDPNCL----TCCLNKTCKYFKSSKKI-KNIKNKM-----  226
c_000002992548_1        146  IKRVFSRLF--NANL--------EKNEKRIKRI----TKE-QLY---------TDRCGDLAEALMEFGAVICKPSNPMCN----ICNLKANCYFFKNKTSV-LVNKKRR-----  225
c_000004056751_1        110  VKRVFSRLLLINTSN--------QNIDNEIKKI----TYK-LPK---------IDRNADFAEALMEFGAVVCKPQNPLCK----ICHLQKYCKFFNSENYI-SLNKKFS-----  191
c_000002655634_1        141  VKRFFSRLFLIDVSS--------QNFENEIKKI----TNK-LPK---------VNRNADLAEALMEFGAVVCKPKNPLCK----ICNLKKYCKFFNGENYT-LPNKKFF-----  222
c_000004232403_2        140  HRRVISRIIGIKNLSR--------HNKNRINNY----LKQ-LVA---------IDRPGEINQAMDIGSIICKPKEVFCD----FCPVQFSCKACVSSKPLEYPNKIYN-----  222
6u7t.after.N146.pdb     141  VMRVLSRLFLVTDDIA------KPSTRKRFEQI----VRE-IMA---------YENPGAFNEALIELGALVCTPRRPSCL----LCPVQAYCQAFAEGVAEELPVKMKK-----  225
Consensus_aa:           l.RVhsRhh.lp.............pph..h....s...hhs..........ppssp@sQAhM-lGthlC..ppP.C......CPl...C.t........hP..........
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Conservation:                                                                                  5   5
sp_P17802_MUTY_ECOL     226  -----QTLPE-------RTGYF----LLLQ-----HEDEVLLAQRPPS-GLWGGLYCFP---QFADEESLRQ---------------------------WLAQRQIA------A  281
sp_P83847_MUTY_GEOS     232  -----TAVKQ-------VPLAV----AVLAD----DEGRVLIRKRDST-GLLANLWEFP---SCETDGADG----------------KEKLEQ----MVGEQYGLQ------V  294
c_000003652391_2        233  -----QPKPT-------RYGIV----YLARD----AHGAWLLERRPDK-GLLGGMLGWP---TSDWNDAPG-----------------------------DQPPFA------A  286
c_000000031207_6        224  -----RTTEQ-------QWWAA----AVVV------CGDQVLVWIGDG-ELLAGHRGTP---LARLSGSGL----------------DADKMIG----KEFKRLGLD-----GA  286
c_000000430975_4        234  -----RKKQR-------RYGVV----YWIER----RDGAISLRQRPEN-GIFGGMTEIP---GSLWKASPL--------------------SRH----SIHAQAPIP------A  293
c_000001007148_4        232  -----RDVTD-------VFELL----VVMR-----RGKRLLLIQHPDG-ERWGGMWGFP---ALSRDRALARS-------------RFDERLIE----SEVADWGCD------V  297
c_000001092848_3        244  -----AAKPS-------RRGAA----FVAVR----ADDAVLLRRRPES-GLLGGMSEVP---TSGWSAGSD---------------GEDGP---------SAASFP------A  302
c_000001151029_1        191  -----TKKRK-------RYGVV----YWIER----RDGAIVLRQRPEN-GIFGGMTEIP---GSLWQAAPL--------------------SRH----SIHAQAPIP------A  250
c_000001797282_1        253  -----AKGAL-------RRGAA----FVAIR---SGDEAVLLRTRPPE-GLLGNMAEPP---GSAWEPDYD----------------VAAAL---------LDAPLD------A  312
c_000001803648_25       236  -----PVKPT-------RYGIA----YIARR----GDGAWLLERRPDK-GLLGGMLGWP---GAQWGDQAV-----------------------------DKPPVS------A  289
c_000002078955_4        236  -----PVKPI-------RYGIA----YIARR----TDGAWLLERRPDK-GLLGGMLGWP---GAEWGAKAV-----------------------------ENPPVS------A  289
c_000002106160_4        224  -----QSLPW-------QNVLT----GVIW-----KRDSFLIIKRAGY-KHLNNLWELP---GGRTLTKNN----------------PHPQLEE----LLKNKFKLE------V  286
c_000003607531_2        237  -----KVKAV-------RKGVV----FLALM----SDGSLFLRRRPEN-GLLGGMMEFP---STVWLERSL--------------------SPK----EICEAAPFK------T  296
c_000003872363_4        232  -----PAKPI-------RYGTA----YIARR----HDGAWLLERRPDN-GLLGGMLGWP---GSEWGEEAV-----------------------------ENPPFS------A  285
c_000004820107_1        241  -----KPKPQ-------KRGHV----FWISD----DSGNILFIRRPEN-EMLGGMLGLP---TTEWDIRP-------------------------------IKLP------Q  291
c_000005774797_1        223  -----RSKKK-------WNAFA----YVIYN----ADGNIGFVRRDKD-ALLGGMWALP---TSDWSTSPV--------------------FQP-----------PI------L  275
c_000005867021_3        236  -----PVKPT-------RYGIA----YVARR----PDGAWLLERRPDK-GLLGGMLGWP---GAEWGAQAV-----------------------------EKPPIV------A  289
c_000004546210_2        229  -----KKKRT-------ESMYF----LMIVF----DGKKLFLEKRKPK-GIWGGLWSFP---QIQLADDPQI-------------------------WCEQQFTNN------I  286
c_000004551008_5        229  -----KKKRT-------ESTYF----LMIIF----DGKKLFLEKRKPK-GIWGGLWSFP---QIQLADDPKT-------------------------WCEQQFTNK------I  286
c_000002511148_4        224  -----KSKPT-------KKVVW----LLPQG----PSGEVLLEKRKPK-GIWGGLWAFI---EAEKKNELEL-------------------------ELSRRFKTD------C  281
c_000000577378_2        212  -----KSKPT-------KKVVW----LLPQG----PSGEVLLEKRKPK-GIWGGLWTFI---EAEKKNELEL-------------------------ELSRRFKTD------C  269
c_000000598175_2        235  -----KVMPE-------KQAIM----VILKN----SKQEVFMQKRPPV-GIWGSLWCFP---QFEKRAFAEE-------------------------WLKSNYGKL------I  292
c_000000754627_3        232  -----KAVPK-------KSCHQ----LIIK-----HNDKVLMEKRPNS-GIWGGLFGFF---EFNEYSELET-------------------------FLAQQGLK-------  286
c_000000811118_1        223  -----KEKIE-------KKIDW----ILIK-----TKNKVLLKRNKEK-GIWQNLWVLP---EKDFFSSKKI-----------------------------SKI------L  273
c_000000899687_2        227  -----ISSKK-------IEVSA----GIII-----KNKKVYIQQRVKN-GLMGGLWEFP---GGKREQGES----------------PEECLKR----EIKEELRVN------V  289
c_000001176522_24       248  -----KVMPV-------RKVCW----QVSLS-----QGCVWLVQNPAR-GLWGGLWVLP---EGNR-----------------------------------------  288
c_000001345122_1        202  -----KIIPT-------KEKFF----LILVT----EAFQVALVKRPEK-GIWGGLWSLP---EFDSVQEIDL-------------------------FLDGNEMVAT----LK  261
c_000001515736_6        220  -----IKRSS-------ESWWL----LLAVD----ARRRVWLQKRPQA-GIWAGLYSPP---VFEDYAALQS-------------------------YAQAAWSQD-----GT  278
c_000001682161_6        223  -----KTIPT-------KERWF----LVLLT----KEGNIGLIKRPAK-GIWGGLWSLP---EFDEVKLLEN-------------------------FITDNNVKCL----LK  282
c_000001923643_29       230  -----LKRSS-------EAWWL----LIAVD----GQGRVWLERRPQQ-GIWAGLYAPP---VFNEREALEQ-------------------------AAARHWPAA-----RS  288
c_000002030994_5        224  -----KEMPE-------KHTVM----LILQN----DQQEVLMQKRPPV-GIWGGLWCFP---QFDKTALAEE-------------------------WLDDSFGMS------L  281
c_000002038721_4        226  -----SQKKE-------KNINW----LLIA-----SKDKVLLKRNVNK-GIWQNLWLFP---EANSLNYEK-------------------------FNL------L  275
c_000002523527_15       251  -----AAIPE-------RSTVM----LIVRH-----GRDVLVQLRPSS-GIWGGLWSLP---EMPVDTVPFD-------------SEAAEESA----LELARAYGS------P  315
c_000002608528_2        224  -----KVMPE-------KQTVM----LILKN----GKQEVFMQKRPPA-GIWGGLWCFP---QFDNHENVLE-------------------------WLDNNYAVS------F  281
c_000002961510_2        227  -----VPSKK-------IEVSA----GIII-----KNKKIYIQQRSKN-ALMGGLWEFP---GGKREKGES----------------PEECLKR----EIKEELRVN------V  289
c_000002979152_8        232  -----KDKRI-------KQSVM----LYVSN----ENADVYLERRSST-GIWGGLWSLP---EFETNVKAND-------------------------WLVKTFALE------S  289
c_000003248034_1        247  -----RKKKR-------VMTSV----IACE-----QGGQILMKQRSVNARLMPGFWELP---EVEGPFLNQ----------------ADL-------------ARWS------L  301
c_000003294679_3        229  -----KTNPL-------KTTNM----LFLRF-----EDRIYLERRPSS-GVWGGLWSFP---EISKDCSVSD-------------------------WYQSRFGAY------P  285
c_000003333364_1        225  -----KPAVP-------VDVAS----GVLV-----HDGYIFIQKRPED-GVWPGLWEFP---GGTVEKGET----------------PAQCVVR----EFQEELEWD------V  287
c_000003402697_5        220  TEKPKKRKHR-------IEVGI----ACIW-----REGKYLVQARPKG-KSFEGSWEFP---GGKREKGEN----------------FRECVKR----EIQEEVGID------V  287
c_000003492925_4        212  ------VVPT-------REENI----MLYV-----YNDKLSLTQRKG--KFLHGLWGFE---NTEMPL-----------------------------------------  252
c_000003766664_5        239  -----RPPKK------IALVAC----VIRD-----EQGAVLLTQRPEK-GLFGGLWEVP---LKEVDETPS----------------KAGVGR----LVKETVGLT------A  301
c_000003948186_72       226  -----NNIPT-------RHTQM----LLIEN----TAGEVWLEKRPAQ-GIWGGLWSLP---ELATGQNAIT-------------------------YCEQELQIA------V  283
c_000005057120_4        225  -----KVLAV-------KQLIF----LMLQD----QQTQFLLEKRPAS-GIWGGLWSFP---EFQSFAEIKS-------------------------WCLEN-DIA------M  281
c_000005590109_5        225  -----KSLPV-------KKITF----LMCQD----QQGRILLEQRPKT-GIWGGLWSFP---EYESLANIQS-------------------------YCFLEK-GIP------I  281
c_000006063368_2        224  -----KTKTT-------KHVHW----LIPYS----HKGRFLLQKRLNK-GLWEGLWVFI---EKEKEEMLKK-------------------------ECKSIFQIK------D  281
c_000000467631_2        204  -----KTLPV-------KNIMF----LMLKD----ELGLVLLEKRPAT-GIWGGLWSLP---EFETLSEIQS-------------------------FCQQN-NFA------I  260
c_000000788235_1        225  -----KTLPE-------KKAVF----LLLIN----INHEIFIIKRPPS-GIWGGLWSLP---QFDTYLQAQQ-------------------------WYEQYFSTH------L  282
c_000001187176_11       226  -----KRIPV-------RQCLM----PLLVN----PQGDIWLQRRPDS-GLWGGLWCPP---QLDSREQLDA-------------------------LVIAQ-GWQ------A  282
c_000001660697_64       231  -----KAIPE-------RAATM----VIALH-----GETVLLQRRPQR-GIWGGLWSLPLVGEMDDALDAHP-------------------------LDADTVRQAALAYGTV  296
c_000003378864_2        238  -----KPVPH-------HHIGV----GVIWK-----DDQVLIQLRPPE-GLLGGLWEFP---GGKQEPAET----------------IEETVRREIREELGVD----------V  300
c_000003910742_3        226  -----KVIPE-------KNAVM----LMLIN----NKDEVFIMKRPSP-GIWGGLWSFP---QFDDYDQAQA-------------------------WHENYFGFP------C  283
c_000004116181_2        192  -----KKQAT-------RTCVF----ILIVN----AKQEILLQKRSPV-GIWGGLWSLP---EVEDTIEAIH-------------------------WCSSR-KIT------V  248
c_000006223903_1        226  -----RPRPR-------REVVM----IMIKD----SRDRVLLERRASS-GIWGGLWSFP---ECPVEEALEP-------------------------WCRMRFGAG------I  283
c_000001029068_2        199  -----KPKPV-------KTTAM----LIFDN----GKGGVYLQKRPAK-GIWGGLWSFV---ECSATDKAIT-------------------------KTAHNFHPQ------A  256
c_000001128125_11       234  -----KDKPV-------KRAFF----LLLLN----SERQVLLRKRPQK-GVWAGLWSLP---EFDSLQGFKE-------------------------SIESQTTV------I  290
c_000001595844_4        225  -----IKRKT-------RYFYY----FVFR-----KGSDYYIQKRTKK-DIWQSLYEFP---NIESKTPLN----------------DNEVKIV----FQEKYPRIK------F  287
c_000001719155_2        229  -----APSTK-------IAVSA----GVLF-----RRNRIYIQKRKAE-GLMGGLWEFP---GGKFESGES----------------PEQCLHR----EIKEELGVT------I  291
c_000002363038_6        234  -----KNKPV-------KQKWL----LLQKN----SAEQVFLYKRPQK-GIWAGLWSLP---EFDSYDRAVD-------------------------FLNGEIHRH-----DA  292
c_000002561400_1        190  -----KEKPV-------KQAWF----VVQLN----AKNQIRLIKRPQK-GIWAGLWSFP---EFCSVTDAKT-------------------------YLQTQTPG------L  246
c_000002566325_2        226  -----AVIPK-------RDTVF----AIMEN----NNGEILLEQRPSS-GIWGGLWCFP---EFSSPLIREE-------------------------MIKRKYGFN------I  283
c_000002598045_3        224  -----KSKLT-------KKVLW----LLPQG----PSGEILLEKRKEE-GIWGGLWSFI---ETEKKAELEL-------------------------ALSRNFDSN------I  281
c_000004188575_41       226  -----KTIPT-------RQSHW----LILLT----DDRRVLLSKRPEK-GIWGGLWCFP---SFDDTHTL-------------------------EQHIDNT------K  279
c_000000240049_1        224  -----KILPV-------REKRL----LIIRN----KQGHYLMEKRPPS-GIWGGMWSLP---ELTLDKSIAE-------------------------SIERNWQLS------V  281
c_000002139456_2        224  -----KILPV-------REKRL----LIIRN----KQGYYLMEKRPSS-GIWGGMWSLP---ELTLDESIAE-------------------------SVERNWQLN------V  281
c_000003535614_2        226  -----KILPI-------QQKRL----LIIRN----EQGAYLMEKRPPT-GIWGGLWSLP---ELALEAPIIT-------------------------EVKNTWQII------V  283
c_000002657784_4        223  -----KQKPF-------KTVFW----LVVMN----KNGKVLLKKRNNL-GVWKGLWSFL---ESENIQDLGK-------------------------ECLTMFEKR------K  280
c_000003800129_15       221  -----TPKKK-------VFLNY----EIYK-----RDGCIYMIKNMDL-GFWNELWMPP---YKIVE-----------------------------------------  262
c_000003716781_1        216  -----PTPID-------VELEM----FLVH-----DHGRVLLRRRDEG-GRMAGLWELP---TREVAAAELRLF----------PAELELEL-----------------  272
c_000001556689_3        216  -----LVKA--------VTEYA----AVIE-----DNGRLLLFRGQRP-SLVSDMWEFP---TLDSRLADTSSRSLTEKPASSHSRAQEAKLSR----YIKEQLGWS------V  293
c_000001834452_1        196  -----INWKE-------VHLLY----GVAS-----CPSGVLLEERKS--GWNQGLWEFP---SVPYDQKEE----------------PDL----AWREFQPQR------G  253
c_000004255004_2        226  -------TKA-------VQWPL----TLAK-----WRSRILLHRRPDK-GILASLWELP---TPENLPAEL----------------IDETL----------------  274
c_000000605438_3        219  -----IKKPI-------RKRAL----IIYA-----KNDKYALAQNEE--RLLSGLWGFK---QEEDFEF-----------------------------------I  262
c_000000581237_15       219  -----KKKPI-------RKRTL----IIYT-----KDDKYALSQNSE--RLLSGLWGFK---QEEEFEF-----------------------------------S  262
c_000002529579_2        219  -----IKKPI-------RKRTL----VIYH-----KANKYALAQNEE--RLLSGLWGFK---QEEDFEL-----------------------------------L  262
c_000006097838_1        208  -----APVSK-------RRFIG----FIIA-----WHGNVFVQRRPSG-EVNSGFWEFP---NWLVDDTQ----------------VQPAD----LASTKLALG------Q  267
c_000000583727_6        234  -----AATTN-------RRFIG----FLIA-----WRGKVFVQRRPSG-EVNAGFWEFP---NWQVNDAE----------------LKPAA----LAKRELKLG------Q  293
c_000005807640_2        236  -----AATTK-------RRFIG----FLIT-----WHGKVFVQRRPSG-EVNAGFWEFP---NWEV-DAA----------------ATADQ----LAKRELRLG------Q  294
c_000000134878_1        207  -----ETKPH-------YDVAV----GIIW-----DKKKLLITKRKEE-GLLGGLWEFP---GGKIKKNEK----------------IESAIKR----EIKEELSIN------I  269
c_000005136725_3        222  -----HTKPH-------YDVAV----GIIW-----DKQKLLITKRKEE-GLLGGLWEFP---GGKMKKNET----------------IKSAIKR----EIKEELSIN------I  284
c_000001684786_4        222  -----RKTIA-------VELEA----GIIR-----RGKRCLLERSESF-DFLEGLWLFP---LSLPGD----------------GGIAA----RLGQALETD------I  279
c_000002971826_23       232  -----RKTVA-------VELEA----GIIR-----RGKRYLLERNEDL-DYLEGLWSFP---LARPDS----------------GGIAA----RLRKRLATS------I  289
c_000002040695_2        233  -----TKKPC-------RWGHF----FFLVHIPSSGSPVSVLLEKEHG-PLLEGLWRPL---TQSWVHKKT----------------EAH--------------FPY------K  290
c_000003263657_32       239  -----KKERP-------HHDHVA----GVIK-----LDDKFLIIQRPLD-GLLGGLWEFP---GGRRRESES----------------LTEGVTR----TILESTGLT-----V  302
c_000005989041_2        237  -----KVKPV-------RKGIV----FLALM----SDGSLFLRRRPET-GLLGGMMEFP---STNWLERRI----------------SPE----EICEAAPFK------S  296
c_000002391082_2        234  -----KKQKP------HYQVAC----GVIC-----KGDRLLLAQRPSA-GMLGGLWEFP---GGKQEEGET----------------LQQCLQR----EIAEELAIE------I  297
c_000003839553_2        241  -----KKKKP------HYQVAA----GVIS-----KGRQLLIAQRPAE-VMLGGLWEFP---GGKQEEGET----------------LEECLVR----EIREELGIE------I  304
c_000003996707_2        231  -----KPTRP------HYEVTA----GVIW-----KGSKLLVAQRPSD-GMLGGLWEFP---GGKREPGES----------------LQECLRR----EIREELTID------I  294
c_000002044706_1        214  -----TKVTF-------VQRVA----LFLEK-----QDGSFLLVRRPAD-GLLAGMWELP---GRELGDGEAPE----------------RLASTLCG---EFGLTIRPD-------  270
c_000006007075_1        224  -----KKKID-------KYFLI----KIYK-----KNKKILLIRNTKF-NFLKNFKIFP---MEEIPKP-----------------------------------  267
c_000002094036_4        200  -----KKIIN-------KFYLA----TLYK-----NQNQILLIKNDKF-KFLKNLLIFP---MREITYP-----------------------------------D  244
c_000002762689_1        224  -----KKNNN-------KYYLL----KVYK-----DKNKYLLIKNTKF-NFLKNFSIFP---MQELSKP-----------------------------------  267
c_000005516980_2        224  -----KKNIN-------KYFIL----KVYK-----KDQRYLLIKNTKF-NFLKNLRIFP---MEELFQP-----------------------------------  267
c_000003920004_1        233  ----KKSRMK------KYTRAY----IIIN-----GSEEILVRRRASK-GMLPSMLEVP---NDKWVTEKK----------------LLVRD------SSVSLFK---------  292
c_000004008511_2        227  ----KSTKRK------KYSRAY----IFYN-----EKNEILIRKRPSK-GMLASMLEIP---NDNWVINKK----------------SLVTD------TIAIKLK---------  286
c_000004481347_1        234  ----LEQKKI------KFTRAY----IIMN-----KKNEVLVNRRKSK-GMLASMLEVP---NDEWVDVKK----------------KLKRH------DVYKDLS---------  293
c_000003787733_3        226  -----KIIPH-------KEIVA----GIIW-----QRDKFLITKRPEN-ALLGELWEFP---SAEIQFNET----------------PIGALRR----QISKDFNIY------I  288
c_000000754657_2        226  -----KVIPH-------KEIVA----GIIW-----QREKFLITKREEN-ALLGGLWEFP---GGEIASSET----------------PVGALRR----QIKEKWDID------I  288
c_000000990943_3        226  -----KVIPH-------KDIVT----GIIW-----RGKKFLITKRSEN-ALLRGLWELP---GGEFESNET----------------PIEALRR----KIKEECDID------I  288
c_000004474996_2        225  -----KVIPH-------KEFVA----GMIC-----QGNKFLITKRNEN-ALLGGLWEFP---GWEIVSSET----------------PIGALRR----KIKEEWAID------I  287
c_000001463500_11       213  -----SIKNI-------KHFIA----AIIW-----YNKEFLIMKRPVN-SMLGGLWELP---NTQIDLKIS----------------DDIQLFN----YIKNSLNFT------I  275
c_000001286181_5        218  -----VTKTQ-------ESFHW----FIYQ-----KNNKVMLCKNPDE-GIWPNLWVFP---KRELFQT-----------------------------------K  262
c_000001293628_3        223  -----PRVRE-------ASYAV----LVTLN----PVGELLMVRRPPD-GLLGGLWEFP---AVEMEAEERNPWGGELR-VGDSLHGRCSARLE----DLGVQLSAR------R  301
c_000001535696_8        222  -----TKVRN-------RYFNY----IIPIAEADDGSRKTLLNQRKGK-DIWQNLWQFP---LIETKREID----------------LEEVKK-----RLCEVMHLAD---DPQ  291
c_000001614067_2        233  -----KEKEE-------RYGLF----FYLQN----KDGAVLFETNKSS-GLLANMDVLP---SIGWYEESN----------------RFKKSPK----FNKKKPNFL-----GM  297
c_000001765289_1        198  -----KKRKP------HYTIVA----GIIW-----RDNTFFIQRRPEK-AMLGGLWEFP---GGKVEEGES----------------LEAALKR----EIKEECGVV------P  261
c_000001961666_1        213  -----KTIRK--------GKCY----IIKRL----NDSKFLFIRNPAK-GLLGSMLLFP---TYGWLHSDH----------------DDYVK----SMIEKFIKN------I  273
c_000002018097_4        221  -----KPIPT-------KEYVA----VFIS-----YNDHIFINQRSED-GLLGGLWELP---MIEVFENSN----------------KIKALKT----YAKNKLGLS------I  283
c_000002843512_36       213  ------VVPT-------REQNI----MLYF-----YNDKLSLTQRTG--KFLHGLWGFE---AIEVPL-----------------------------------  253
c_000002930199_1        220  -----RKPKI-------RYFNY----LFIK-----KNQQFLIQQRGTN-DIWKKLYELP---LIESKEKLN----------------REKIMA------HQYFKPFK------I  280
c_000003033795_2        214  -----KKIPI-------YDIAV----SVVE-----YQNKILITKRQNK-NFLPGLWEFP---GGKIEKKET----------------ATQAIIR----EVKEETNLT------V  276
c_000003888107_2        215  -----KTIPT-------IKVST----CIIR-----KQNKLLILQRPID-TMLGGLWEFP---GGKIQNNES----------------RKKAVIR----EVMEETNLA------I  277
c_000004013286_6        242  -----PKIQA-------VREVA----VIVR-----KGKRVLVVQRPSD-GRWAGMYDFP---RFECSFRSKE--------------KIRQLMVR----GVGQQTGVP------I  306
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c_000004852258_1        202  -----KTIPT-------KTIAA----ALVN-----HGDNIFITKRPLK-GLLGGLWELP---NIELVNGEI----------------PEDLLKI----KFADQFGLT------I  264
c_000005254087_1        230  -----KRTPHYDVVAGIIWQNG----VSPG-----EGGRFLIAQRPLN-GLLGGLWEFP---GGKQEPDET----------------LPQALER----EIREEMDMM------I  299
c_000005603677_1        227  -----LKIKK-------RYFYY----FVWRG----TNGGYYLRKRQEK-DIWQGLYEFP---MIELDKPLK----------------TSELKT-----LWAHWELEE------L  289
c_000003283462_3        213  -----KNIPH-------YDVVV----GMIW-----KEGRFLIQKRENQ-KHLSGLWELP---GGKIENNER----------------SELALIR----EIKEECDFE------V  275
c_000002687221_1        227  -----VSRPH-------HNVAV----GLIW-----KDNRILISKRNAS-GLLGGLWEFP---GGKIRSGES----------------GSSCVVR----KTQEILNVL------V  289
c_000003347358_19       244  ------VVPT-------REENI----LVRV-----YDDKLSLTQREG--KFLHGLWGFE---SVEVPH-------------------------------------------  284
c_000002701031_2        222  -----LKIRK-------RYFEF----LIIN-----ENEKILIQKREK--GIWKGLYQFP---LIENSVKKSE----------RDIENSKS----WKKLFKHNS------I  285
c_000000582753_3        230  -----RKSTN-------RYFTY----YYIS-----DSNHLYIKKRVGK-GIWESLYEFP---LSENLIAFE----------------NEVLDK-----QIFKNSIND------F  291
c_000001713769_5        222  -----NAPKI-------IHFNY----LVLL-----DSDHMICMDRIKN-GIWKNLFQFP---MIESKKELN----------------KTQVLSN----EIFKSIAPIS---NSD  287
c_000004369364_1        225  -----IKKRT-------RYFYY----LVFK-----EKDLFLIQKRTQK-DIWQSLFEFP---NIEGMEELN----------------ESEIREA----IQKKYPNLK------Y  287
c_000006057486_30       226  -----IKIKK-------RHFNF----IVI-N----NNQHTYIHQRKDN-DIWKNLYQFP---LIESKHEIDTN------------KVIKSSIF-----QDFFKNNSY------T  291
c_000005494072_10       222  -----IKIKK-------RYFNY----LVVLS----KKNKITLNKRINK-GIWQNLFEFP---LIETVETITIK------------TLVEHEYL-----EKIINKKQL-----VD  289
c_000001742634_3        225  -----IKIKK-------RFFNY----LILIT----KDNKTIINKRINK-DIWLNLFEFP---LLETKTEINEN------------KLVKNDFF-----QKLILNDTT-----IN  292
c_000002826998_2        225  -----IKKKT-------RYFNY----LVLNF-----GKNTWVQKRTKK-DIWQNLYEFP---LLETDKIIS----------------QKELMKS----ELWKESVSDF----DF  289
c_000003159439_6        222  -----IKIKK-------RYFNY----LVIIN----SQNKIALQRRKHK-DIWKGLYEFP---LIETLTAIDIE------------QLLETEEF-----HQIINEDQA------I  288
c_000004887214_1        227  -----LKIKK-------RYFNY----LILKT----KNEETLVKQRKGK-GIWQNLYEFP---LLETEKEINYL------------ELIDHSIF-----KLETEKEINYL------D  293
c_000005037037_2        225  -----VKVKE-------RFFYF----LVLN-----IDNTTYIEQRQNN-DIWKGLFQFP---MIETQQLIN----------------KPKELEK----LITNKINVR------F  287
c_000001059964_1        217  -----KKVKI-------LYRGC----AVIL-----FQDGLILCKRNQP-GLLEGLWEFP---SVELGKGDN----------------LRKALQE----HLLEELGKP------F  279
c_000002498472_1        226  -----KKIIP-------IDMAT----GILI-----HNGMLFIQQRLPD-DVWGSLWEFP---GGRMELKET----------------PEQTVVR----EFLEETEFK------V  288
c_000004615912_3        215  -----IRKKK-------RYFHY----FISHF-----EGKILLRKRIQK-DIWQNLYEFP---MLETKDEELK----------------ISRVQKT----QLWQQLFHGNEKVKVL  284
c_000004766858_2        228  -----PEITR-------IEKLT----ALVQ-----NGDCWLVRKRPEG-GLMGGLWEFP---TWEELPSENS----------------AAAWLTQ----QLHEDYGVA------A  291
c_000002747260_18       232  -----KPKPT-------RHGIA----YLGRR----ADGAWLLERRPDK-GLLGGMLGWP---GGDWAETAT-----------------------------EAPPID------A  285
c_000005371561_38       232  -----KPKPT-------RRGIA----YLGRR----ADGAWLLERRPEK-GLLGGMLGWP---GSDWAESPP-----------------------------EASPPCP------G  286
c_000000169465_2        234  -----KALPE-------KSTYM----MVAQ-----FNSQVYLEQRPST-GLWGGLYGFI---EVSSIEEGIE-----------------------------QLAKRGIS------V  289
c_000003254110_11       234  -----KALPE-------KATFM----MVAQ-----FNSQVYLEQRPST-GLWGGLYGFI---EVSSIEEGIE-----------------------------QFKKRGVN------V  289
c_000004750284_20       230  -----KVTPV-------RQTYM----LIPM-----FGQQVFLEQRPQS-GIWGGLYGFL---EAQSAEEGIS-----------------------------LLAQRGIE------V  285
c_000000141782_15       235  -----REKPL-------RRTRM----LLIVD-----GGRVLLERRPPS-GIWGGLWCPP---ECPPEVDVES-----------------------------YCRHELGLE------V  291
c_000002717847_8        224  -------KKD-------VYLEF----SLYK-----DGPKYFLAQTEAL-GFWKKLWMPP---VKIVN-----------------------------------------  263
c_000004054799_1        215  ------QKPT--------VKLN----FILPH----TKNEILMHKKQAS-EYWESLWIPI---DGDEVKIK-----------------------------------------G  259
c_000006126673_1        241  -----KARPQ-------RYGYV----YWISD----DDGNILVHKRPEK-GLLGGLYGLP---TSSWETDIE------------------TVEH----PIFISEQIK------P  301
c_000000339186_3        199  -----IRYED-------KQIDV----YLIT-----RGPRYLVQQRPEG-GVNAGFWEFP---NSDSGNKYS------------------APK-------------------  247
c_000003029168_2        230  -----VPLQSREE-VGIVIWAG----KPYN-----ADSLIFIVQRPPT-GLWAGLWEFP---HDEQTTDEP----------------HNAAALR----IAEATVGPT------M  298
c_000001863436_1        246  -----ARQKP-------VAARTAVVTSGGA-----EGRRFLMVKRPAD-GLLGGLWEFP---TVEAAGDVA----------------PTAQAVS----RLLARLTGGP---VPA  315
c_000002830137_4        239  -----TQTEH-------VVEAA----VVIR-----RGSRVLLRRHEDR-ERWAGLWDFP---RFPFDPDATN----------------NGHQQLVD----KSRELTGFE------I  303
c_000004612302_3        232  -----VRTTD-------LYQLL----LVIR-----RDDRLLLIQHPER-ERWGGMWGFP---ALSRTGPLLKS----------------RIDELQIA----SEAAEWGCD------V  297
c_000001933926_1        217  -----VARPT-------RHGVV----FWATR-----GDGCVLLRRRPEH-GLLGGMMEFP---STEWRECPW----------------RIRSAV-------KAAPFA------A  276
c_000003136334_1        229  -----KRKPT-------RYATV----FWLLD----GRGNVLLRRREEK-GLLGGMMEVP---STDWLEENT----------------PTKENS--------MFAAAA------V  288
c_000005849454_9        234  -----KKWVD-------LEMVF----LVHR-----VGVRVGLQKRTS--GWSPGLYEPP---SAICESFATANE----------ASSAHESTAH----SLAREHR-------A  299
c_000001169194_1        234  -----VPARE-------ISFLL---VILQT-----EEGEVMLVQRPPE-GLLAGMWAFP---EQELAKPLD----------------CAATSRD---RAIELAMSLGA---EVV  301
c_000002786947_2        234  -----APARE-------IFFLL---VILQT-----EEGEVMLVRRPDK-GLLAGMWAFP---EQELHKPLD----------------CSTTSRD---RAIGLAMTLGA---EVV  301
c_000001279808_52       220  KKTTKRRKHR-------IEVGI----ACIW-----REGKYLVQARPKG-KSFEGSWEFP---GGKREKGES----------------FRGCVKR----EIEEEVGLN------V  287
c_000000358065_2        224  -----KKIIN-------KFYLA----TLYK-----HDDQVLLIKNDKF-KFLKNLLIFP---MKEISQS-----------------------------------------C  268
c_000003745941_1        218  -----KIKQE-------TIDRA----WVEQ-----GGKLLLHRANDKS-RRLSGILELP---TLDVMGY----------------------SAT----ALMK------------  268
c_000004187032_4        224  -----KKNKE-------KYFIL----KVYK-----KNQKYLLVKNTKF-NFLKNLAIFP---MEELSKP-----------------------------------------  267
c_000006067315_3        224  -----KFNKI-------KYFEA----NIYQ-----NQNKYLLIKNSKF-KFLNNLLIFP---MKEVEKN-----------------------------------------K  268
c_000005543774_1        186  -----IESKT-------YDIFC----YLRK-----NKKQIALTKNNDL-GFLKKFNLPE---IKATSKK-----------------------------------------N  230
c_000006211484_1        208  -----IQSKN-------YDIFC----YLQK-----NKKQIALTKNNDL-GFLKKFNLPK---IKETSEK-----------------------------------------N  252
c_000002718976_3        227  -----IKSKN-------YDIFC----YI-N-----TKKQIALTKNNQI-SFLKDFNIPE---IKE-TNNVSN-----------------------------------N  272
c_000002992548_1        226  -----KKEQK-------YNVYC----YLKK-----KKKEIALTKNKHL-SFLSNFNIPE---TKIETSNNKK-----------------------------------N  273
c_000000456751_1        192  -----IKEKK-------INIYC----YLNR-----YKKEIALTKNKNL-SFLNNFKMPE---IQMVVANNNLK-----------------------------------K  240
c_000002655634_1        223  -----TKEKK-------FNVYC----YLNK-----YKREIALAKNRNL-GFLYNFKMPE---IQMEVTNNNLK-----------------------------------R  271
c_000004232403_2        223  -----KKIPT-------RNLAA----ALIE-----YGDYILISKRPIN-GQLGGLWELP---NIELKNGKS----------------PEKSLKE----SINNHYGFT------I  285
6u7t.after.N146.pdb     226  -----TAVKQ-------VPLAV----AVLAD----DEGRVLIRKRDST-GLLANLWEFP---SCETDGADG----------------KEKLEQ----MVGEQ----------  283
Consensus_aa:                ......................h.....hh.........phhl.pp....shh.shh.hP.....p.....................................p...........
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Conservation:                          6
sp_P17802_MUTY_ECOL     282  DNLTQLTAFRHTFSHFH---LDIVPMWLPV----SSFTG-------------------CMDEGNA---------LWYNLAQPPSVG--LAAPVERLLQQLR--TGAPV------  350
sp_P83847_MUTY_GEOS     295  ELTEPIVSFEAFSHLV---WQLTVFPGRL----VHGG--------------------PVEEPY---------RLAPEDELKAYA--FPVSHQRVWREYK--EWASGVRRPD-  366
c_000003652391_2        287  DWRTLRGEVRTTFTHFH---LILRIRVAEL----PDDI----------------------TPAVG---------FLLSKHAFRPSD--LPTV----------------------  338
c_000000031207_6        287  ELIGHADRFQHAITYRK---LFIHPLVYRY----QGE----------------------PAGV---------ETIPIDGGDR----LPALHRKSIAAAR--ELLLEVFR---  352
c_000000430975_4        294  DWKPHPGRVYHSFTHFD---LELKIMTINS----EVT----------------------PEGT---------FWCRKEEVKAQA--LPSLMHKIMTHMY--KA---------  354
c_000001007148_4        298  EFQEWMDEFQHGVTRFR---LHLQPIVAMW----RSGSW--------------------EGPVTA---------EWVGIDELATRP--LSVAGRRVATRVE--RILRHNGAQA-  370
c_000001092848_3        303  PWEPC-GTVTTFTHFT---LALSVYRARV----ADMP----------------------APAGS---------WWCAPGLLAGEA--LPTVMKKVVEAAI--SGATKARRPIG  373
c_000001151029_1        251  DWKPQPGLVYHSFTHFD---LELKI-----------------------------------------------------------------------------------  272
c_000001797282_1        313  RWKRLPGLVRHGFTHFP---LELTVFVARV----ALATP--------------------APAGT---------RFTPRSALDDEP--LPGLMRKVLAHAF--DPKPEPEKKPR  385
c_000001803648_25       290  DWQDPGAEVRHSFTHFH---LRLSLRISVV-----GNA--------------------KPTAG---------HFIADADFDPET--LPTVMRKAYRIAR--TKFDGDANG--  358
c_000002078955_4        290  DWQVLNAEVRTTFTHFH---LRLSLRVATI-----GNK--------------------PPATG---------DFISKNAFDPET--LPTVMRKAYRLAC--TQFDDN-----  355
c_000002106160_4        287  TIKNKIGEIRRYSHFG---ITLSGFHCRL----INGSK------------------PEVNRPF---------RWIRLNEINQYA--FPKANHKLFNLMQ--E----------  351
c_000003607531_2        297  QWVKIAGTVNHAFTHFQ---LQLQVRVAVV----NDQT--------------------QISGG---------IWVDRDRLDEFA--LPNLMKKVWAFAS--KRIQPLPRLDC  368
c_000003872363_4        286  DWQRLDAQVRTTFTHFH---LRLSLRIVVA-----KNV--------------------IPTVG---------MFIPSDKFDPDD--LPTVMRKAHALAD--AAFDHG-----  351
c_000004820107_1        292  ESEQTNLSVKHSFTHFD---LIL-------------------------------------------------------------------------------------  311
c_000005774797_1        276  SEWKGIGQIRHIFTHID---L---------------------------------------------------------------------------------------  293
c_000005867021_3        290  DWHVLGAEVRTTFTHFH---LRLMVHVATV-----NEN--------------------SPDVG---------KFIADDCFDPAD--LPTVMRKAHKLAL--GSFVGD-----  355
c_000004546210_2        287  LWVDSWEPFKHSFSHFD---LYIHPIQIRM----DGQFM------------------NTAYKSKT---------QSFSLNQLDSLG--LSAPVKGLVNKLY-------------  351
c_000004551008_5        287  LWVDPWEPFKHSFSHYD---LYIHPIQIRM----DGQFM------------------NTAHTPKT---------QSFSLNQLGSLG--LSAPVKGLVNQLY-------------  351
c_000002511148_4        282  LNIKKLSKVKHSFSHYN---LEAIPYLAKL----ESNK--------------------KYKNS---------VWVDYKNVESLG--LPAPIKKTINQLT--KP---------  344
c_000000577378_2        270  LNIKKLGKVKHSFSHYN---LEAIPYLATL----GSNK--------------------KYKNS---------VWVDYKNVESLG--LPAPVKKTINQIT--KP---------  332
c_000000598175_2        293  QESIALAEIKTTFSHFH---LYIQPLIINV----ETPIE-----------------LGVMEDNES---------LWYNITTEFNGG--LAAPVQTLINITK--NIEKEIENGTN  369
c_000000754627_3        287  SDLNEVAPFTHVFSHFE---LTINPHVLNI----EKAPD------------------VVNDKQL---------VWYPLDQSIEVG--LAAPTKKLVKQIT--AIV--------  353
c_000000811118_1        274  PVEEDFSSFEHHLSHKK---LLISVKVLTI----KNKDR-----------------LSIDRRIF---------NWVNISDSVNMG--IPKPVKDILKKYD-------------  338
c_000000899687_2        290  VSLNKVMTIKHTYTQFR---VTLTVFNCKL----QKKQI------------------RPDGCEQW---------KWVSLSKLKKYP--FPAANVKIVKYLT--EK---------  356
c_000001176522_24       289  --GEALSAFRHTFTHFH---LDITPYLWHT----HSDD--------------------MPQQAG---------QWLPLAQALKMN--IPAAVKRVLQEVI--EYEQNR-----  354
c_000001345122_1        262  EQAVI----KHTFSHYH---LRMKPMM---------------------------------------------------------------------------------  281
c_000001515736_6        279  RSWQDLPGFLHVLTHKD---LHLHPVLVPV----DQAYA-----------------ALVSEADES---------CWADAAAWAELG--LPAPIRKLLDAEL--A----------  345
c_000001682161_6        283  DSVEILKQLKHTFSHYH---LMMNPVMLQI----ERE--------------------AIEEV---------SWFELENLNGVG--LPTPIKKILRESF--A----------  343
c_000001923643_29       289  ADWTELPAFLHVLTHRD---LHLHPVRVPV----HGSGP-----------------DAAMADGQG---------CWADARAWADMG--LPAPVRKLLDAQF--AG---------  356
c_000002030994_5        282  SEADKLSKIKHTFSHFH---LHIQPLIINT----ETPLK----------------MGVMEHDDS---------LWYNVTTEFNGG--LAAPVQTLLNITK--DIVKGTQNDTN  358
c_000002038721_4        276  EEKESLPLIVHNLSHQK---LSIFTKKYFL----KNKDR------------------LPIDRRGF---------VWVNKKESVKMG--IPKPVKTILENHL-------------  340
c_000002523527_15       316  ARAYLAGELTHVFTHFR---LLIRAIRVDL----DTVAL------------------EGDAAS---------RWLSLDDLDMLG--TPAPVRKLLEDQA--RFGLF------  383
c_000002608528_2        282  DEAIKLSEIKHTFSHFH---LHIHPLIINM----ETPLK----------------IGVMEHDD-----------------------------------------------  321
c_000002961510_2        290  ISLNKVMTIKHTYTQFR---VTLNVFNCEL----QKKQI------------------RPDGCEQW---------KWVSLSSLKKYP--FPAANVKIVKYLT--EK---------  356
c_000002979152_8        290  PSLQAYAEIEHEFSHFH---LSAKVLRIEL----DSDKR----------------LGVMEETNA---------LWYNMHKDNPGG--FPAPIKKLLSRNL--L----------  356
c_000003248034_1        302  KHPKKLCKFRHSITFRL---YDVSIYTAIL----EGE--------------------LPEPF---------CWVPQRKLATIP--LTTVTRKALAVLS--PNSSDRNDEQN  372
c_000003294679_3        286  LKISRWKTLQHKFSHFD---LKIHLFKAEP----DIAIK----------------NIANNDSA---------LWYKSDSQTRIG--LAAPVKKLLETQF--NIQ--------  353
c_000003333364_1        288  AASETIAVIRHGVTSFR---VTLTAFLLRF----GEPPEGW-------------PEPVLHDATEG---------VWVSFAELDRYT--FPAGHRKLIDQMS--RDMRYAWLV--  366
c_000003402697_5        288  SVRPHFYEELHEFETE---LLLRFHRCQI----QAGEP------------------SPQEEQEI---------KWVSPDDFGDID--FLKTNHKALDKLK--EMRV-------  356
c_000003492925_4        253  CASEYIGEVTHAYTHFK---LICKVYVVDE-------------------------LDAEQN---------SYFTSSKIQKLA--ISKVDDKLILKYL--DTIQTY-----  316
c_000003766664_5        302  RIEGQCSSVRHVLTHRD---MDIAVFALSV----KGREPS----------------PALSTYTGG--------MWIHSPEALEDLG--VGRVTRKILEAAA--THEISGQER--  378
c_000003948186_72       284  DSCTELAPRRHTFSHFH---LLITPLHIKS----SQQTT-----------------HVMEDQQQ---------LWYNMRKPASVG--LAAPVKRLLRQTS--NQ---------  350
c_000005057120_4        282  QSVQHIEKQRHTFSHFH---LDYTAVLVKT----ENRIN----------------NVMESNQS---------VWYKSDKINALG--LPAPIKKLLQNQN--RNEV-------  350
c_000005590109_5        282  KLIKQLDEQRHTFSHYH---LDYTGIMVKI----ENPIN----------------IVMEANQS---------VWYKSTQLKFLG--LPTPIKKLLQNQY-------------  346
c_000006063368_2        282  PSLIQIKKVKHSFSHFN---LEAIPYLVKI----KEKK----------------SIKNT---------VWVDSKNIESLG--IPSPVKKTIKEIR--VP---------  344
c_000000467631_2        261  TSKKQLDGQRHTFSHYH---LDYTPIEVKI----ENPIN----------------NVMEANQS---------VWYKSEQINSLG--LATPIKKLLQNQ-------------  324
c_000000788235_1        283  PSATTESHFSHTFSHFR---LQAQTLLITQ----STPIK----------------RVMEGTPS---------LWYNSNTTFMGG--FPAPIKTIINKLN--EDF--------  350
c_000001187176_11       283  AEPEALSPLRHTFSHFH---LDIQPLLVRV----DAPDR----------------VTESGQ----------LWYNLREPSRLG--LAAPVKKLLQRAE--LNTQADERTSP  356
c_000001660697_64       297  SDVEAAGALTHTFTHFR---LHMHLLHADI----TKP--------------------ATLDDDW---------RWVPLAQLNSVG--LPAPVKLALETLV--QPSLL------  363
c_000003378864_2        301  EVGPLVTQVRHAYSHFKITLHAYHCRLLRG------TPH----------------PKVAVES---------RWVPLNGLDQFA--FPKANKRVLEVLEQPPAMPEVTP---  375
c_000003910742_3        284  ENTEKETKLSHTFSHFR---LHIHALLIRQ----TPPIK----------------RVMEGTPS---------LWYNRSTKFTGG--FPAPIEKLFTRIL--T----------  349
c_000004116181_2        249  KTSEVLPTLRHTFSHFH---LQYTTLVVRT----DNPLN----------------FVMEVDQA---------VWYKAEQIPGLG--LPAPVKRLLDNLI--LNEDNND----  320
c_000006223903_1        284  LFESAWSVLRHSFTHFE---LAITPQPARA----PKRLT----------------RRMQSTGL---------LWYKPGEPF------------------------------  332
c_000001029068_2        257  QVRKKLPLINHSFTHYY---LTI-------------------------------------------------------------------------------------  276
c_000001128125_11       291  ESLVEWNYFRHTFTHYH---LDIYPLVYRLD--EGEVSPVFTIG--------KSQNNDEKASAEM---------NWIELDRLNKGEVGVPAPVNKMLERLS--ETLDK------  374
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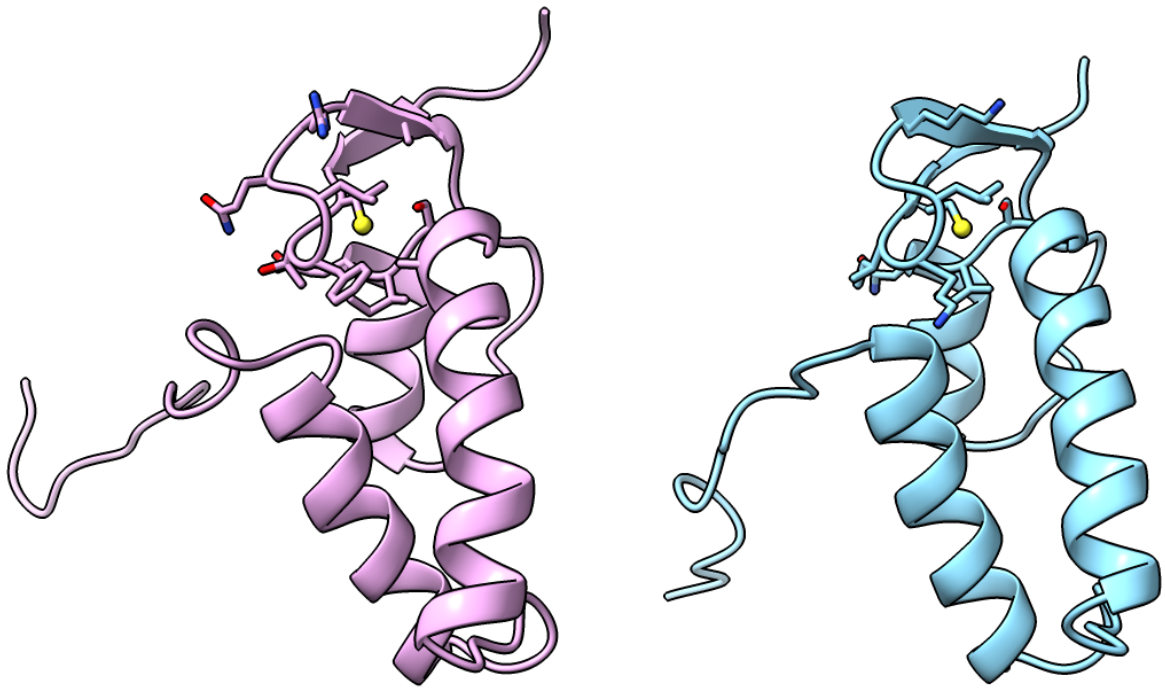
c_000001595844_4        288  NIKNKLGPVTQLLTHQK---VIGQFFDITL----VGE----------------------KDLDI---------APISKVDLQVFP--FPKMMMMYINRIM--A----------  348
c_000001719155_2        292  HIDEKLMTVKRSYTRFR---VILHVFLCRV----LSGRV-----------------SPTHCEEW---------DWVKTEELDRYP--FPAANVKIIKSLR--KNQGRS-----  362
c_000002363038_6        293  HSLSEGEPFRRSFSHYH---LDIFPLIF-------QASTDYEIGQP---SNRVQETPAEYSVAHH---------QWFDINRLKSGEIGVPVPIRKMISSL-------------  370
c_000002561400_1        247  EGLIEWDDFRTTFSHYH---LDIYPLIFDE----RGAPKNTAKI--------ISEPGGVYDAEQG---------SWFDIQLLLDGGLAVPAPVNKLIGRLD--ELVE-------  327
c_000002566035_2        284  KQQKEYKPIKRAFTHFH---LTIRPIHIKI----RIYKD-----------------KDYDTKVL---------KWIHPKTNPNLG--LPAPVLSMLIDLN--NTKRAVLNES-  358
c_000002598045_3        282  SNIKKLKKVKRNFSHFN---LEAIPYLVKV----KKGK----------------------KIKNT---------VWVNFKNVESLG--LPAPVKKTINQIT--MP---------  344
c_000004188575_41       280  TVSKQPAVFRRTFTHFK---LDIHPHLYHA----KQSAA-----------------ENITGQA---------IWYKIEDALTLG--LPAPVKKTLLTLS--KG---------  345
c_000000240049_1        282  NSHNDLAAFRTTFSHFH---LDITPCEVDI----TQSAY-----------------AIAENDRF---------QWH--ADITQLA--LAAPISAIFRENN--SLK--------  347
c_000002139456_2        282  NSHNDLAAFRTTFSHFH---LEITPCEVDV----AQSAH-----------------AIAENDRF---------QWH--ADITQLA--LAAPISAIFRENN--SLK--------  347
c_000003535614_2        284  KKHHDLPVFRTTFSHFH---LDITPCEIEI----KSISN-----------------AIGDDSCY---------QWQ--VDISKLA--LAAPVSAIFNAK-------------  345
c_000002657784_4        281  KDLKTYPDINHSFSHLN---LNAKLFLLKL----NEDIN-----------------YNINKGKY---------VWVDKKGLKALG--IPTPVKNIFEVLK--LNDQTS-----  351
c_000003800129_15       263  ---NNNREIKHGLSHRE---LHLEFLVTSG-----------------------------TPKGG---------KWINKNEFNNLP--TPKPISDKLEQYA--KSFL-------  320
c_000003716781_1        273  LQREEVGELRHSITKHR---IRARVLRAVP----GRDE-----------------------LPEGW---------AWFERSEIEALG--VTGMTSKALTLGR-----ARSR----  337
c_000001556689_3        294  RLGPKLGEIRHGMTDRR---ISCHVYEASL----SGSQTTMAGA------PTGLEKSDHNIPDS---------GWFTIEEVMTLA--LAASARKTLTTLL--DIDLTTAHNPG  380
c_000001834452_1        254  KLGEMMGSARTITRHRR---IQARVHQVEG-------------------------------W---------NGKGAVDPSSVP--LSSLGRKVLSIAG--VLGGLLLLSPD  317
c_000004255004_2        275  QQGDPVALIHHAITDMR---ITAPVYLLQC----KIPV-----------------------KGPEW---------RWVSLANLHAYP--LSSLSTKALSAAR--QHLPTTRKLGG  346
c_000000605438_3        263  ENMTGLGEFKQHYTHFT---LDARVVLLKD-------------------------------KKQE---------NYFTVEEIHDLA--LSGADRKALALLE--KYLQ-------  322
c_000000581237_15       263  KDMIKLGEFKQHYTHFT---LEASVVLVQN-------------------------------KEQE---------RYFRINEIHALA--LSGADRKGLALIE--KHLA-------  322
c_000002529579_2        263  NNMTELGEFKQHYTHFT---LDGSVVLVTN-------------------------------KKQK---------NYFTIDEIHDLA--LSGADRKALALLE--KYLQ-------  322
c_000006097838_1        268  VEPESLCIINHSITCYR---NRLEMFVIFR----TRKPL-----------------LAKLKG---------KWVPLAKLDEIP--LTTAHRKAANQFA--G----------  331
c_000000583727_6        294  AKFQPLCNINHSITRYR---NRLEMIVAEP----KRKPR-----------------LAKLKG---------EWLPLAKLAELP--LTAAHRKASDYFL--KTANSR-----  362
c_000005807640_2        295  AELEPLCTINHSITRYR---NRLEMFVIEP----ARKPR-----------------LAKLNG---------EWIPLAKLDELP--FTTSHHKAANQFC-------------  357
c_000000134878_1        270  KPETIIHQVDHHYSHFS---VTINAINCKY----NGGDI-----------------KLSGPTDF---------KWIHTSKLHSYA--F----------------------  322
c_000005136725_3        285  KPEKLIHQVDHHYSHFS---VTINAINCYY----NGGKI-----------------KLSGPTDF---------RWVKTSRLHSYA--FPKASIKLFNAIK--SIE--------  352
c_000001684786_4        280  ETGEELKAIKHSITFRR---LTLRPRLLET----GSFTI-----------------RGKPSF---------RWALPEELGSVL-PVSSLCLKIADRLS-------------  343
c_000002971826_23       290  EEGAELEAIRHSITFRR---LTLRPRILGA----GSFST-----------------RAKPSF---------RWGRADELGNVL-PVSSLCLKIADRLS-------------  353
c_000002040695_2        291  ASWTLLGQIQHVFTHFT---LKLDLWYTIR-----------------------------TVKTQG---------QWVLLNQMNDYP--LSSLTKKSLAFLV--KNLELEKEQAR  359
c_000003263657_32       303  KTDLVLPTVLHVFTHFK---ITLYGFGCTF----IKGAA-----------------KALTCHDA---------RWVSQTQLADFA--FSRAHNRLIDIMK--KEAAKGQVEMF  378
c_000005989041_2        297  QWVKITGTVTQPFTHFE---LQLQVRVAVV----ENKP------------------KSSEG---------IWVDRNRLDDFA--LPNIMKKVLAFAL--RHNQHFSREFF  368
c_000002391082_2        298  EVGARLTSVDHAYSHFS---ITLHALEARY----LRGEP-----------------QAIGCADW---------KWIVPDELDDYA--LPRADRLIVEFLR--QDGRQLGLFDS  373
c_000003839553_2        305  EAVARLTSVDHAFSHFS---ITLHAIAARY-------------------------------------------------------------------------------  331
c_000003996707_2        295  EVGGLLAKVDHGFSHFS---ITLNLFEAMY----RGGEP-----------------EAVECDDW---------RWIDPAELDDLP--MPRADRRALEELS-EEEGQRVPVLMA  371
c_000002044706_1        271  ----LVVTSEHRFSHRH---VTREATRVYRA-----RVEDE---------------CSGPADASHWSEGL-----RWTCAGDLGKLA--VPEASRKTLRPCLRASRGSLPG----  353
c_000006007075_1        268  --KNLQKTLNTKMSNMN---MNIVIKIMKI----SSN----------------------ISDS---------SWIKETKFNHYT--FPTFTKKIFNSLE--NYL--------  327
c_000002094036_4        245  SLLKSKNKLNLKMSNMN---MNICIDFVKI----KKK-----------------------PKNG---------LWIESSKLENCM--IPTFTKKIFASVK--NNL--------  306
c_000002762689_1        268  --LSSTDIVNTKMSNMN---VGS----------------------------------LPTS---------HWIDPKKLKNYT--LPTFTKKIVRYLE--SNK--------  327
c_000005516980_2        268  --KNFNENLNFKMSNMN---MNIKIQYSKN----NIK-----------------------LPSS---------YWVDQKKLNNYM--LPTFTKKVVKYLE--NNK--------  327
c_000003920004_1        293  KKFTKCKPILYSFSHFD-------------------------------------------------------------------------------------------  309
c_000004008511_2        287  NKLKLKGFVDYSFSHFD---LETEVFFTKV----KKDY----------------------FANQ---------KWIKKNNIKISG--LPTVMKKIIEVAL-------------  346
c_000004481347_1        294  VKYKRIGKITYSFSHFD---LDIGIYKSKV----VKKK----------------------FKNF---------NWIKSNKIDTSG--MPTVMKEIVKKSL--KI---------  355
c_000003787733_3        289  NIGKKIGHVKHAYSHFR---ITLTLYQCKT----NRSVK-----------------NIYKEY---------KWIIPTEVNNYP--FPKSNHKLFK----------------  348
c_000000754657_2        289  NIKKKVGNVKHAYSHFK---ITLTLYRCQS----QGSVK-----------------SNNKEY---------RWITPAEIKYFP--FPKSNHKLFDVLN--SNGWNV-----  357
c_000000990943_3        289  NIEKEVGYVKHAYSHFK---ITQTLFQCQT----QESVK-----------------SINKEY---------RWITPIEVNNYP--FPKSNHKLFTILN--SDGWNV-----  357
c_000004474996_2        288  NIKNKVGYVKHAYSHFK---ITLTLYQCQT----QESVK-----------------IMNKEY---------RWITPSEIQYFP--FSKSNHKLFNLLN--SNGWNV-----  356
c_000001463500_11       276  QIQNKICTIKHSYSHFD---IRVSLYHCRI----NDINI-----------------KNMKNV---------CWIRPKDINNYA--FSKSNHKLFKILN--TNGWTI-----  344
c_000001286181_5        263  QNINELPSFKHSLSHKD---FHISPRIINI----PDDME-----------------TDDKKT---------IWIEKNKIAKLG--APKPVLDIVKKIL--NQNDKGLL---  333
c_000001293628_3        302  VRFSPLPEVRRTFTHLK---AVYRPMLVIG----TRCEG-----------------SNPDSEH---------KWVLPDQVEDLP--LPVAQRRILDLAW--AALEGC-----  371
c_000001535696_8        292  VLRYNHKIIVHKLSHQQ---LHTHFWIVVI----SNELD----------------------------------GSILLSEIENYP--VPVLIANFINTFK--NSYFWPNNSIR  359
c_000001614067_2        298  KWKILDQNLNHHFTHFK---LNCSLAIATI----NDENAL---------------VNDLSKSSY---------RFVQKTNINELA--LPSLIKKILNALK--NNEILDF----  371
c_000001765289_1        262  SIKKRIGAVDHAYSHFS---ITFHGYHCIE----NGEK-----------------------------------------------------------------------  292
c_000001961666_1        274  NRILSKNVVRHEFSHFK---LELEVYYIET----RFP----------------------QITHG---------EWLKTKQAGKQ---LPSVMKKVLDNI-------------  331
c_000002018097_4        284  DILNCFDKVKRSYSHYK---LLVTPFECEV----LNA----------------------DSRIG---------RWIRVGEIDKYA--FSKVNHKLFNAIG--IKNY-------  347
c_000002843512_36       254  CASKYIGEVTHAYTHFK---LIIQVYVYDE-----------------------------LDAEKN---------SYFSSQEIQKLA--ISKVDDKILNLFL--NSIECD-----  317
c_000002930199_1        281  SNIKNKYTIEHNLSHQK---LKISFWDVDA----KNIN----------------------TQKGF---------EKITLDNISNYP--FAKPLEKYFQTQH--LIT--------  344
c_000003033795_2        277  SNPIFLGNITHKYSHFG---VNISFFISFP----KSIKS-----------------FNSSQEY---------RWIRIKNILNYP--LPKANHKMLDILK--KL---------  342
c_000003888107_2        278  KNPNYLGQIKHQYSHFK---VLISLFLADV----NDISS-----------------LKPNQNY---------KWTTRKQLDKFA--LPKANYKMLEILD--KLN--------  344
c_000004013286_6        307  QIGNKIGQIRTVVTRFR---TTVDCYEAHF----SNIGKPNRSKNDISVNTLQKEQQEAGSLWHM---------QWATRKQIDSFP--MSVPARRVIRLLA--DFD--------  392
c_000004852258_1        265  KVGHKLGMINHTFTHFK---MNVLLYNCQA----KDQP----------------------LSKNTE---------KWVSFSDLDQYA--FSKANHKLFKLVE--LRND-------  330
c_000005254087_1        300  RTDRFLTSIKHAYTHFR---ITLHAFHATH----LRGEP-----------------QHIGVADH---------AWVTLADLDKYA-------------------------  351
c_000005603677_1        290  VWALISGQEQQQLSHQK---IIARFLWPKQ----PLPQ----------------------ALSADW---------ELVDAQSWPNYP--LPRLIAQYWEKKQ--LAFF-------  355
c_000003283462_3        276  VPKKKIESIKHKYSHFS---ITLTAFHCKI----KNGAK-----------------VPSKQST---------RWITPGEIGDYP--FPRANHKLFNQLE--NFYSNG-----  345
c_000002687221_1        290  APVAFVKQIKHAYSHFS---ITVDAYSCTF----RGGRP-----------------RALGADY---------RWIYPHETGKYA--FHSAGHKLFDKIL--ETAS-------  358
c_000003347358_19       285  CASEYIGKVTHAYTHFK---LICKVYVYYE-----------------------------NHPEEA---------DYFSEEEIQKIA--ISKVDEKIVNLYG--NFLKYD-----  348
c_000002701031_2        286  QILKISATIKHQLTHQT---IFARFWHIDT----KIQ-----------------------KVENS---------KYISKKELKNYP--ISRLLEKYLENIG--MI---------  347
c_000000582753_3        292  AIRKVHLKIKHVLSHQN---IYAKLIEVEV----LDVI----------------------RLENY---------IKVEKEDFESYP--IHRLMEKMMEQVG--K----------  353
c_000001713769_5        288  IILFNSSPIIHKLSHKT---IYAKFWILPV----NGSNS-----------------------------------NSIKFSDVNKYP--VPRLIEKFLDKFN--YKHF-------  348
c_000004369364_1        288  KIKHKSAPSKQLLTHQK---IIGIF-------------------------------------------------------------------------------------  309
c_000006057486_30       292  ISKYNQKQIVHKLTHQH---LFTTFWIINT----NTCSK-----------------------------------NTVNWQNLSNFA--LPTLIQNFVDDFK--K----------  349
c_000005494072_10       290  VSLFNPKPIIHKLSHQH---IYTSFWIVNY----NGSVS-----------------------------------NTISIDELKKYP--VPVLIDNFIKDFE--M----------  347
c_000001742634_3        293  PYLFNEKTVIHKLSHQH---IYTKFWIVKL----DVNLE-----------------------------------QTIPLNSIEKYP--VSTLIQNFIKEFN--F----------  350
c_000002826998_2        290  QIKKISLPFRQNLTHRK---IIATFFEIDF----MDKFS-----------------NKNNNY---------IKIERKNLSKFA--FPKIIDLYLKDNS--LNLAMK-----  358
c_000003159439_6        289  TSLYSDNIKAHKLSHQH---IFSKFWIVET----KQNIL-----------------------------------NSILIEDLEKYP--VPKLIQNFIEKFD--NIKLNPATK--  354
c_000004887214_1        294  LQLLTPKVVVHKLSHQH---LYIKFWEIRV----PTFRS-----------------------------------ATISWQELLKLP--FPIVIFKFIREFL--ETSMPNFDTPL  361
c_000005037037_2        288  KVKKRSKNFTQRLTHQK---LNAIFIEIDL----KTK-----------------------INKQF---------INTDINNLSKFA--FP-----------------------  336
c_000001059964_1        280  QLGKEIHTLEHRYTSFR---TRVSFFTATV----HVLPE----------------------SSQKRV---------RFVKTSEVE-------------------------  326
c_000002498472_1        289  EIVEKITNTVHHYTRYK---VTLHCFLLRL----KKGSDT---------------DPVLHAAQDF---------NWVPFKALQEYA--FPAGHRKLITYL--------------  355
c_000004615912_3        285  QTKK----YQQELTHQK---ISAYFWEIN-----LNVAP----------------------VNIYDF---------ILIDSKNLAKFA--FPKIIAYLYENNS--LNL--------  345
c_000004766858_2        292  RIGPELFKMEHDYTRFH---ATLHCRLCTV----AEIPS-----------------VLPDNA---------QWLTLGEISALA--LPRVFQRLRKRLL--DEVSA------  359
c_000002747260_18       286  PWRDPGAEVRRTFTHFH---LRLALRVADL----PASA----------------------TPRTG---------QFIPHHAFRPGQ--LPTVMRKAYDLAS--ATFLGN-----  352
c_000005371561_38       287  NWRTVPGEVRRTFTHFH---LVLELRRADL----PEDC----------------------TTMRG---------QFLAPGGFRPSD--LPTAMRKAFDLAR--DG---------  349
c_000000169465_2        290  DETRTLEGFRRTFSHFH---LDITPVVAVV----NSAPS-----------------KRVAETAF---------RWFSLNEPIEVG--LAAPTTKIIQQLM--R----------  355
c_000003254110_11       290  EETKTLETFRRTFSHFH---LDITPVVAVV----NSPPT-----------------KRVADTAF---------RWFSLGEPIEVG--LAAPTTKIIKQLI--G----------  355
c_000004750284_20       286  EEIQQLDEFRRTFSHFH---LDITPLIAVV----NSTPQ-----------------KRVAENES---------RWFLLDEPIEVG--LAAPTTKIIKTLA--K----------  351
c_000000141782_15       292  TRPQAWPGLRRTFTHFQ---LDIQPVRLRL----TAAPA-----------------RLMDGDRH---------VWYNVESPDARG--LAAPVARLLQGLA--LEDTRA-----  362
c_000002717847_8        264  ---KFNAANKHALSHRH---IHFRFKQIAN----LPT-----------------------GMKG---------EWFSKKDLANIA--LPKPISDKLLQDG-------------  319
c_000004054799_1        260  NQKYSKIKVNHPLSHLN---LDMKIKTFKI----DKKCD----------------------------------LDSN--L-----------------------------  296
c_000006126673_1        302  KPYKQLPFIMHVFTHFS---LKLYLCKTSE----FICDN-----------------VPEEH---------KWVFISTLEDIG--FPSVFEKVKKII--------------  362
c_000000339186_3        248  -NGQPFASARHSITRYR---IQLNAHLIHA-----------------------------KRGKG---------MWCTSDEIRSLP--FSSAHLKLLGKLP--HRLDK------  308
c_000003029168_2        299  QKLTKLVTITTVMHTR---TTLHVFEGML----DLETS-----------------TMQEASSR---------LWVRLDGLHKHP--FSSPQHEIIKTLD--KEAALQLAGLV  374
c_000001863436_1        316  TPRVELGEVVHHFSHIR---MTITAEHRVA----SSEQVRL--------------RSDDEVGQEA---------RWVSAVELVDLP--MSAAMRKIEGLYR--ELGSGAI----  391
c_000002830137_4        304  EPVRQLTTIQHGVTRFQ---ITLQCHLAQC----VSGRK-----------------RGPHL---------SWRMPHELEHLP--LSVTARKIGRLVA--KLAD-------  369
c_000004612302_3        298  EVEEWLQEFRHGVTRFR---LHLQPVVASW----RAGHW-----------------IGPVVA---------EWVEIDELTARP--LSVAARRVADGVQ--AGQRCGGASG-  370
c_000001933926_1        277  AWRELPGAVRHSFTHFH---LELQIMTVET----NEEVEG----------------------------------------------------------------------  309
c_000003136334_1        289  DWQMLSGKVRRTFTHFH---LEL-------------------------------------------------------------------------------------  308
c_000005849454_9        300  KLGAYQGQVRRTITHHR---IRAHVYAAEG-----------------------------------Q---------APTHLEDPTQVP--LTGLARKVLDRIT--SA---------  354
c_000001169194_1        302  GVASALAEVQHRFTHLQ---ARYRPWVVPV----AKL-----------------------LNGEGN---------VWMTPGEPVDFP--IPVAQHKVLDALA--ECGATLGSSES  373
c_000002786947_2        302  GHASALAEIQHRFTHLQ---ARYRPWVVPV----AKL-----------------------LTGEGS---------MWMTPGEPVDFP--IPAAQHKVLDALA--ECRATPDSLES  373
c_000001279808_52       288  SVRSHFYEELHEFETE---LLLRFHRCQI----QAGEP-----------------QALENQEL---------KWVSPDEFDSVG--FLKTNHKALEKLK--SMRV-------  356
c_000000358065_2        269  SLSKPDHKLNLKMSNMN---MNISIDFSNN----KKK----------------------LKNG---------LWVEKTKLENHM--IPTFTKKIFASVK--HNL--------  330
c_000003745941_1        269  RGNTPIVTRKRGISNSQ---ITEPIWRLSD----NSEA-----------------DHAEM---------EWIDLEQIKKVT--LSGPHKRWINEIL--TDFNKQK----  336
c_000004187032_4        268  --KNFNENFNFKMSNMN---MNIKIQYLKN----LKK----------------------FPSS---------YWFDKRRLDNYM--LPTFTKKVVKYLE--KN---------  326
c_000006067315_3        269  FNTSLNKKINIKMSNMD---MKIILNKKNK----LIK----------------------IKNS---------YLVDKKNIKDLI--LPSFTKKIFNSVS--NYL--------  330
c_000005543774_1        231  KNWKFLCNYKNSISNKK---LNLNLYYKFS----SRI----------------------PSKY---------NWYSL-KKNKEF--IPTFTKKIFKQIT--HLY--------  291
c_000006211484_1        253  KNWRFLCNYKNSISNKK---LNINLYYKFT----SKI----------------------PSEY---------NWYSL-NKNNEF--IPSFTK-------------------  303
c_000002718976_3        273  QNWIFLKNYKNSISNLK---LNINLYYKFS----KKI----------------------PRSF---------NWYSL-KENKEF--IPSFTKRIFRQVS--TLF--------  333
c_000002992548_1        274  NGWIYLCNYKNNISNIK---MNINLFYKFI----KNK----------------------PKKY---------MWYPIDRRSNEF--IPSFTK-------------------  325
c_000004056751_1        241  RGWYYLCSYKNNISNIK---MNIDLFYKFT----KKI----------------------PKKF---------NWYSIDRSNQEF--IPSFTKKIFKKIA--KVYS-------  303
c_000002655634_1        272  REWNYLCNYKNNISNIK---MNINLFYKFT----KNK----------------------PKKF---------DWYSVDKSSQEF--MPSFTKKII----------------  326
c_000004232403_2        286  EVGKNIGLIQHTFTHFK---MNITLFCCLL----KYDQ----------------------KIDSSA---------KWVRLFELDQYA--FSRGNHKLFQLYQ--KENV-------  351
6u7t.after.N146.pdb     284  ELTEPIVSFEHAFSHLV---WQLTVFPGRL----VHGG----------------------PVEEPY---------RLAPEDELKAYA--FPVSHQRVWREYK--EWAS-------  349
Consensus_aa:                ........hp+.ho+bp...l.hp.h.h.....................................@h..pph.phs..hs...p+hhp.h.............
```

1001
1002 **S2 Table. Percent identity matrix**
1003

	***Gs* MutY**	**LCHF MutY 4**	**LCHF MutY 1**	**LCHF MutY 2**	***Ec* MutY**	**LCHF MutY 3**
***Gs* MutY**	100	37.5	31.2	31.2	38.0	33.3
LCHF MutY 4	37.5	100	34.4	33.4	33.3	33.7
LCHF MutY 1	31.2	34.4	100	65.5	37.0	34.8
LCHF MutY 2	31.2	33.4	65.5	100	39.9	34.8
***Ec* MutY**	38.0	33.3	37.0	39.9	100	48.3
LCHF MutY 3	33.3	33.7	34.8	34.8	48.3	100

1004
1005 We were interested in determining how similar the amino sequences of LCHF MutY
1006 representatives were to existing MutY enzymes. We visualized this in the form of a percent
1007 identity matrix that was generated by Clustal Omega.
1008
1009



S3 Fig. MutY gene neighbor YggX

Structural homology is evident for *Ec* YggX (left) and the nearest neighbor to *Thiotrichaceae* MutY (right). The structure solution NMR structure for *Ec* YggX (PDB ID 1yhd) (103) is superimposable to the structure predicted by *Colabfold* for the nearest neighbor to *Thiotrichaceae* MutY, with RMSD of 0.99 Å for 69 pruned pairs selected from 88 possible pairs. The Cys residue critical for function is highlighted with a yellow sphere for the sulfhydryl group

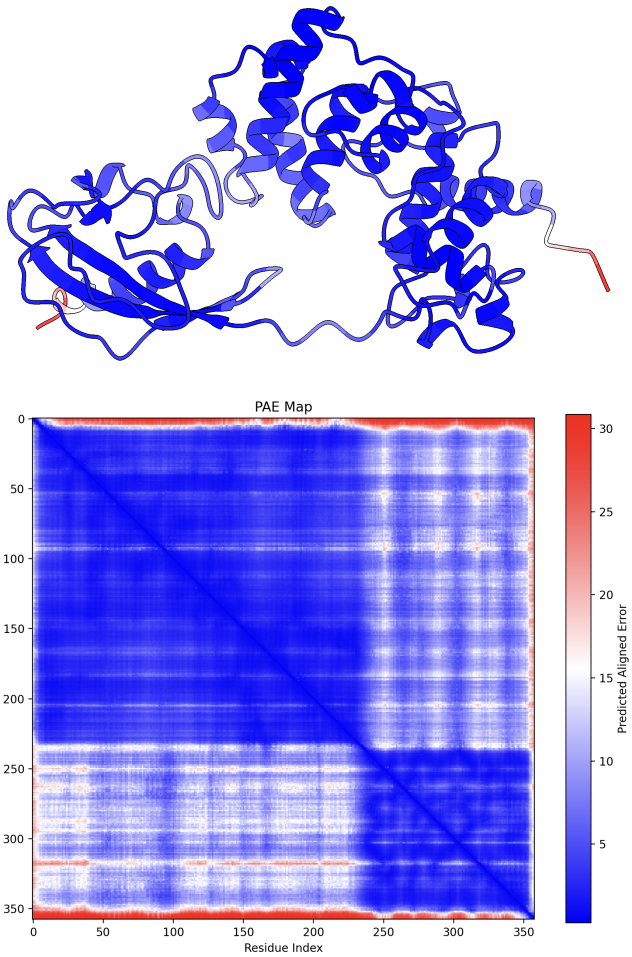
1018 **S4 Table. Metabolic gene identification**

			Marinosulfonomonas [a]		*Rhodobac-teraceae*	*Thiotrich-aceae*	*Flavobac-teriaceae*
			MAG 1	MAG 2			
Cytochrome Oxidases	UQCRFS1	K00411	X	X	X	X	
	coxA	K02274			X	X	
	ccoN	K00404	X	X	X		
	cydA	K00425		X			
	cyoB	K02298			X		
Aerobic CODH	coxS[b]	K03518					
	coxM[b]	K03519					
	coxL[b]	K03520					
Methanogenesis	mcrA[b]	K00399					
	mcrB[b]	K00401					
	mcrG[b]	K00402					
Methane Oxidation or Nitrification	pmoA[b]	K10944					
	pmoB[b]	K10945					
	pmoC[b]	K10946					
Sulfur Oxidation	soxA	K17222	X	X	X		
	soxX	K17223	X	X	X		
	soxB	K17224	X	X	X		
	soxC	K17225	X	X	X		
	soxY	K17226	X		X		
	soxZ	K17227	X		X		
General Nitrogen Metabolism	narG	K00370	X	X	X		
	narH	K00371	X	X	X		
Dissimilatory Nitrate Reduction	nirB	K00362		X	X	X	
	nirD	K00363		X		X	
	nrfA[b]	K03385					
Denitrification	nirK	K00368			X		
	norB	K04561			X		
	norC	K02305			X		
	nosZ	K00376			X		
		MAG Completeness (%) [c]	88.4	88.2	93.7	66.1	44.3
		MAG Contamination (%) [c]	16.4	0.6	1.4	11.8	1.6

1019 [a] *Marinosulfonomonas* MutY contig belongs to two separate MAGs and each are reported separately as MAG 1 and
1020 MAG 2, respectively.
1021 [b] KEGG ID gene not identified in any MAG and not reported in **Table 2** of the text.
1022 [c] Completeness and contamination scores generated by CheckM v1.0.5 as described in Brazelton et al 2022 (35).
1023 A KEGG ID analysis was used to identify the potential metabolic strategies of the MutY
1024 encoding organisms at the LCHF. The full metabolic KEGG ID search is shown above.

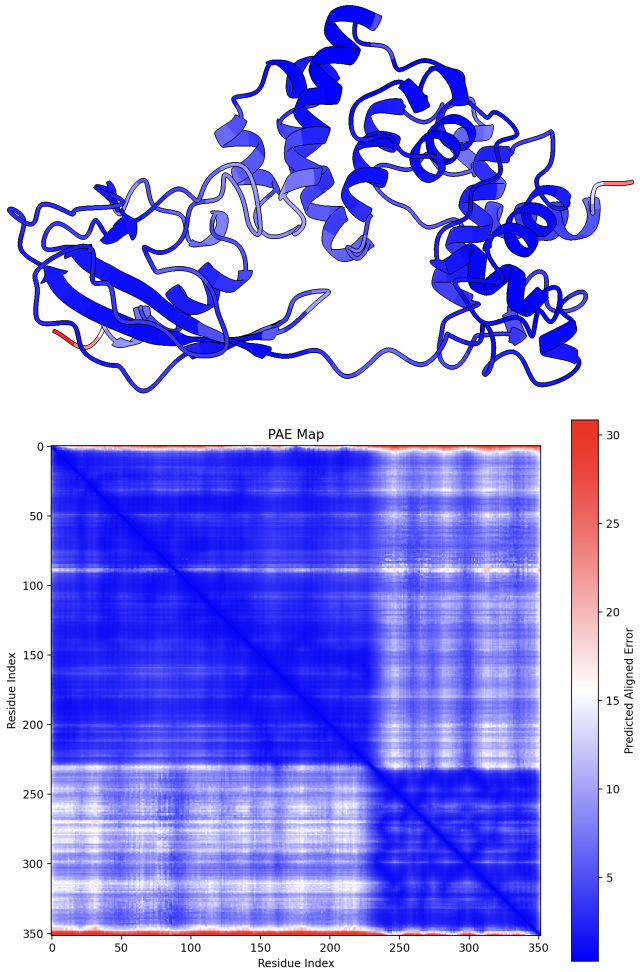
S5 Fig. Colabfold structure prediction pLDDT scores
pLDDT scores represent the confidence in the prediction calculated by *Colabfold*.

Marinosulfonomonas MutY Structure Prediction



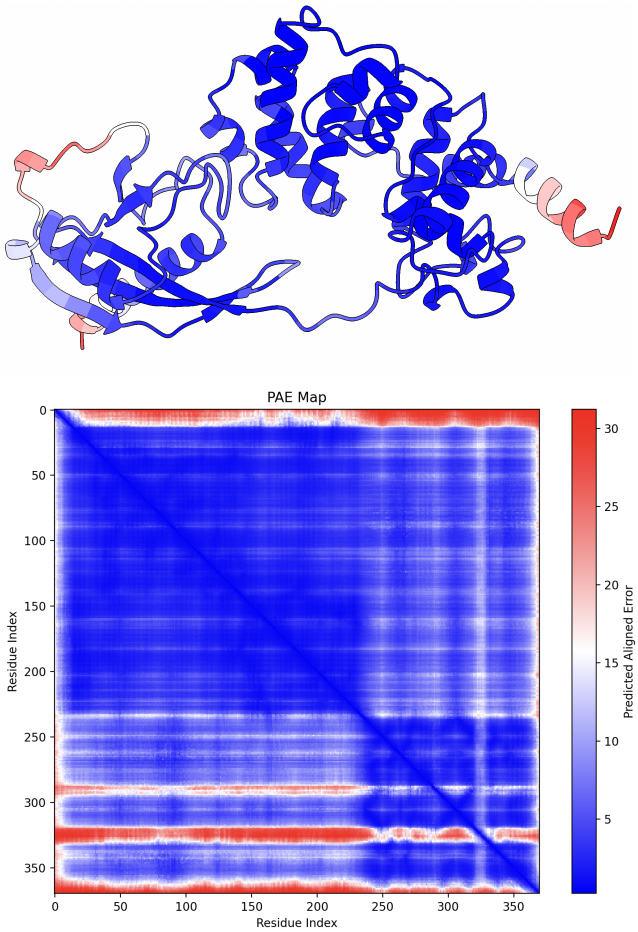
pLDDT score: 93 (±9)
ptm score: 0.75

Rhodobacteraceae MutY Structure Prediction



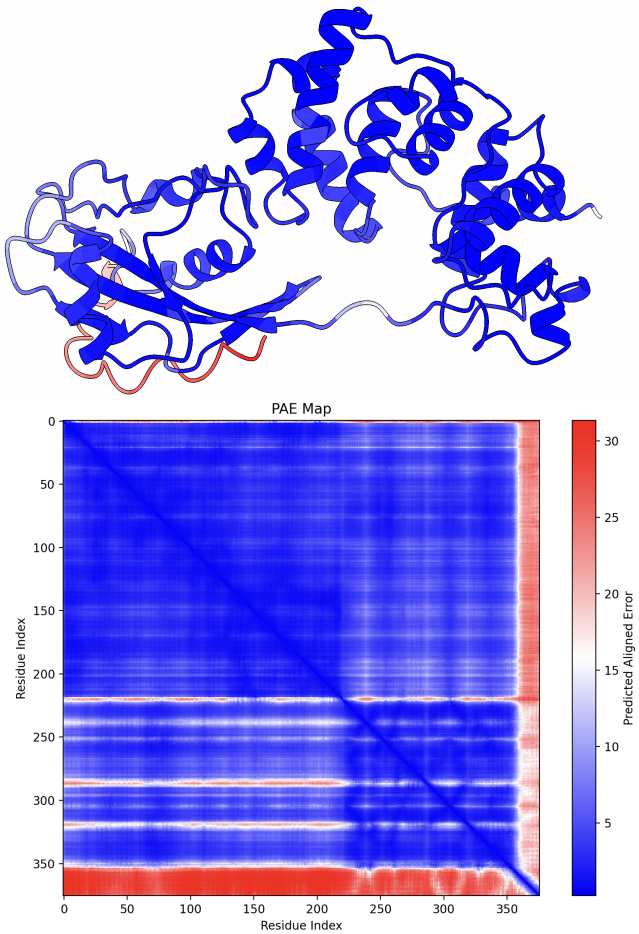
pLDDT score: 94 (±6)
ptm score: 0.79

Thiotrichaceae MutY Structure Prediction



pLDDT score: 92 (±12)
ptm score: 0.83

Flavobacteriaceae MutY Structure Prediction



pLDDT score: 92 (±14)
ptm score: 0.84

1036 **S6 Table. Ligand binding affinity (kcal / mol)**

Adenosine	*Gs* MutY (3g0q)	*Marinosulfono-monas* MutY	*Rhodobac-teraceae* MutY	*Thiotrich-aceae* MutY	*Flavobac-teriaceae* MutY
Mode 1	-7.3	-6.8	-6.8	-7.0	-7.2
Mode 2	-6.9	-6.3	-6.5	-6.9	-7.1
Mode 3	-6.8	-6.2	-6.3	-6.3	-7.0
Mode 4	-6.7	-6.2	-6.3	-6.3	-6.9
Mode 5	-6.7	-6.2	-6.1	-6.3	-6.8
Mode 6	-6.7	-6.0	-6.0	-6.3	-6.6
Mode 7	-6.6	-5.9	-6.0	-6.2	6.5
Mode 8	-6.5	-5.8	-6.0	-6.2	-6.4
Mode 9	-6.5	-5.7	-5.8	-6.1	-6.3

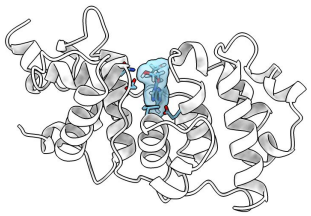
OG	*Gs* MutY (3g0q)	*Marinosulfono-monas* MutY	*Rhodobac-teraceae* MutY	*Thiotrich-aceae* MutY	*Flavobac-teriaceae* MutY
Mode 1	-7.7	-7.5	-7.7	-8.0	-8.0
Mode 2	-6.9	-6.8	-7.6	-7.5	-7.5
Mode 3	-6.9	-6.8	-7.5	-7.3	-7.4
Mode 4	-6.8	-6.8	-7.3	-7.0	-7.3
Mode 5	-6.7	-6.7	-7.1	-7.0	-7.2
Mode 6	-6.6	-6.7	-7.1	-6.8	-7.2
Mode 7	-6.5	-6.4	-7.1	-6.8	-7.2
Mode 8	-6.5	-6.2	-7.0	-6.6	-7.1
Mode 9	-6.3	-6.2	-6.9	-6.6	-7.0

1037
1038 *Binding affinities are reported for the binding modes generated by *AutoDock VINA*. Each mode represents a
1039 predicted ligand pose, which differs by a combination of position, orientation, and rotamer conformation. The
1040 receptor structure was obtained from PDB ID 3g0q for *Gs* MutY and through structure prediction for the LCHF
1041 *Marinosulfonomonas* MutY, *Rhodobacteraceae* MutY, *Thiotrichaceae* MutY, and *Flavobacteriaceae* MutY. The
1042 binding mode representing the starting complex for molecular dynamics analysis is highlighted.
1043

1044 **S7 Fig. Molecular dynamics**
1045 Molecular dynamics simulations were calculated by *GROMACS* with the Amber99SB and GAFF
1046 force fields for MutY complexed to adenosine and OG. Each panel shows representative
1047 structures sampled at 2-ns intervals over the course of the 10-ns simulation along with time-
1048 dependent local interaction energy (top, right-hand panels) and functionally relevant distances
1049 (bottom, right-hand panels). Note that the Y-axis is logarithmic for distance. The adenosine and
1050 OG ligands are shown with all atoms wrapped in transparent surfaces. For the adenosine
1051 complexes, the protein structure was truncated so as to focus on the NTD (residues 8 - 220 in *Gs*
1052 MutY, and corresponding residues for the LCHF MutYs). Catalytic residues are shown: Glu43,
1053 Tyr126, Asp144 and Asn146 in the *Gs* MutY protein and corresponding residues in the LCHF
1054 MutYs. The distance versus time plot for the adenosine complex, tracks potential contacts
1055 between the catalytic Glu (atoms OE1 and OE2) and the hydrogen bond donors and acceptors on
1056 adenosine (atoms N1, N6 and N7). For the OG complex, the iron-sulfur cluster domain and inter-
1057 domain linker were omitted so as to focus on the OG-recognition site found at the interface
1058 between NTD (residues 29-137 in *Gs* MutY) and CTD (residues 234-360 in *Gs* MutY). Residues
1059 that interact with OG are shown: Gln48, Tyr88, and Ser308 in the *Gs* MutY protein and
1060 corresponding residues in the LCHF MutYs. The distance versus time plot for the OG complex
1061 tracks potential contacts between the critical Ser/Thr residues (the amide nitrogen named N and
1062 the hydroxyl oxygen named OG) and the hydrogen bond donors and acceptors on OG (atoms N1,
1063 N2, O6 and O8).
1064

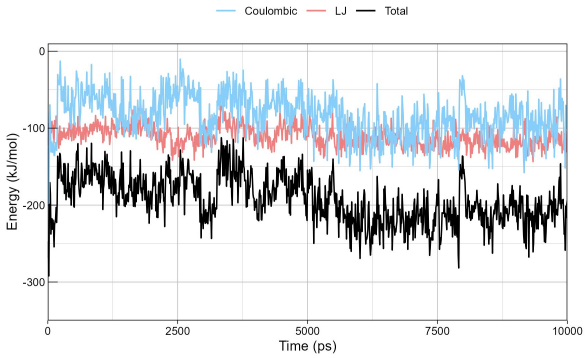
(A) *Gs* MutY complexed with adenosine

0 ns

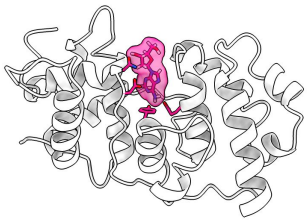


2 ns

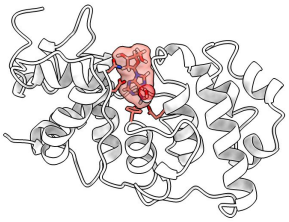
Energy



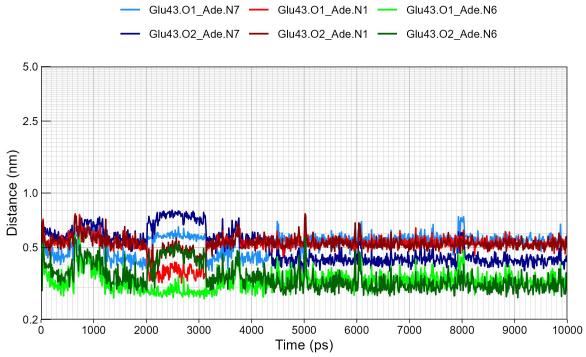
4 ns



6 ns



Distance Adenosine - Glu43



8 ns

10 ns

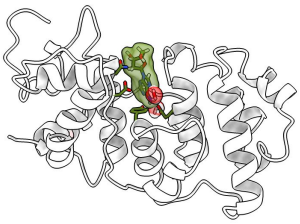
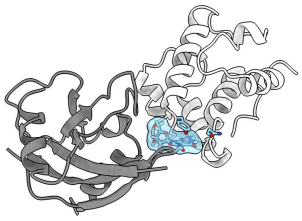


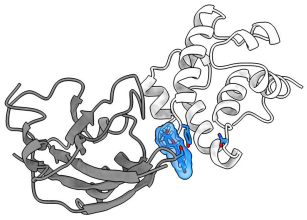
Fig S7. (A) Molecular dynamic simulation for *Gs* MutY NTD complexed with adenosine. The ligand complex was stable for the entire 10-ns simulation, with VDW (salmon trace in the energy versus time plot) and Coulombic (sky blue trace) short-range interactions each contributing. Hydrogen bonds between catalytic Glu43 and the Hoogsten face of the adenine base persisted throughout the simulation. These consistently involved direct contact with N6, as evidenced by close distance (green traces) and inspection of structures. N7 was also engaged (blue traces), with relevance for catalysis, via bridging water molecules (surface with O red and H white).

(B) *Gs* MutY complexed with OG

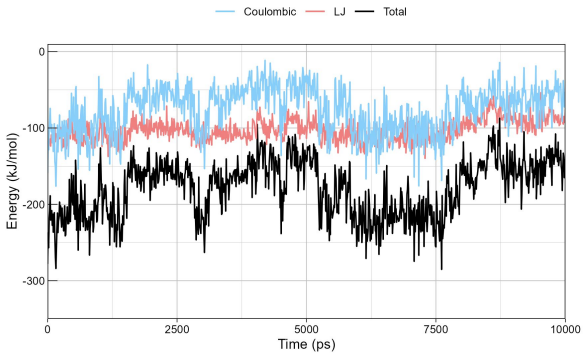
0 ns



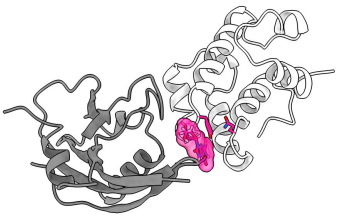
2 ns



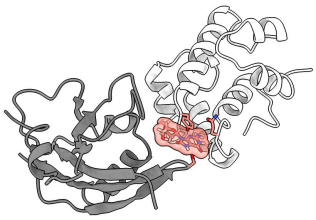
Energy



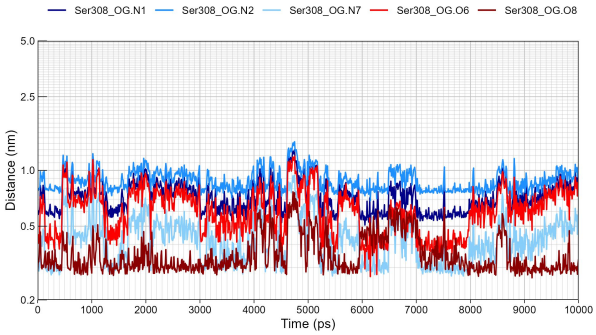
4 ns



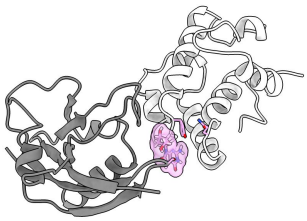
6 ns



Distance OG - Ser308



8 ns

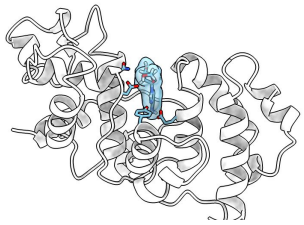


10 ns

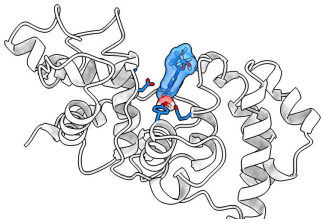
Fig S7. (B) Molecular dynamic simulation for *Gs* MutY complexed with OG. The ligand complex was stable for the entire 10-ns simulation, with the OG ligand bound to a cleft between the NTD (white) and CTD (gray). The functionally relevant hydrogen bond between the amide N of Ser308 and atom O8 of OG was frequently observed as evidenced by close distance (firebrick red trace in the distance versus time plot), and sometimes accompanied by a second OG-specific hydrogen bond between the hydroxyl oxygen of Ser308 and atom N7 of OG (sky blue trace).

(C) *Marinosulfonomonas* MutY complexed with adenosine

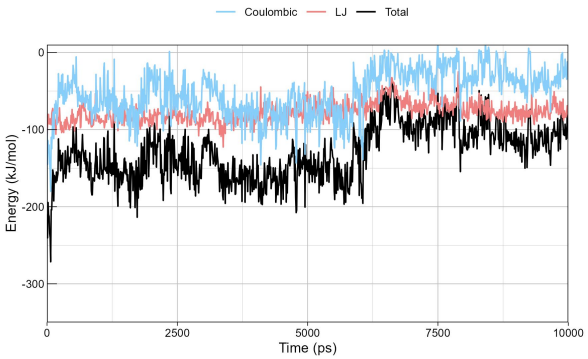
0 ns



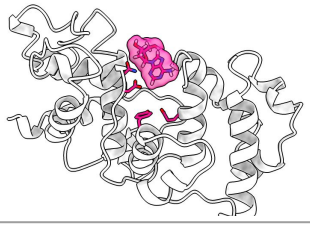
2 ns



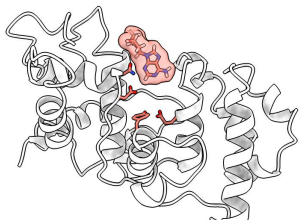
Energy



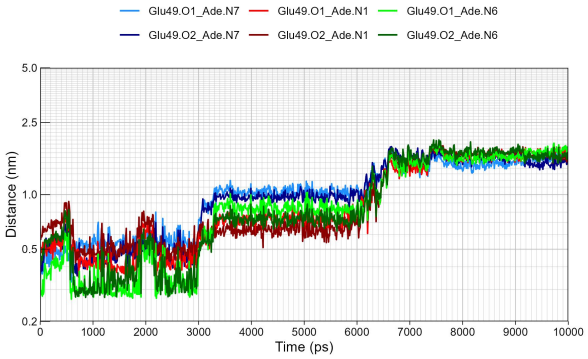
4 ns



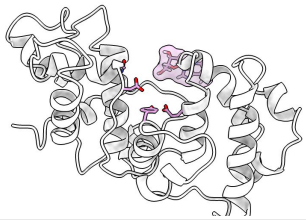
6 ns



Distance Adenosine - Glu49



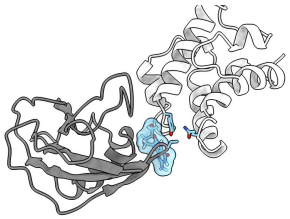
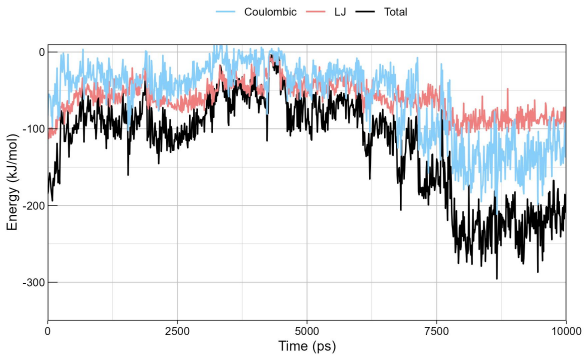
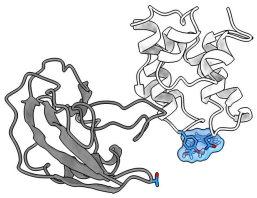
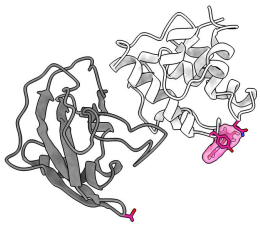
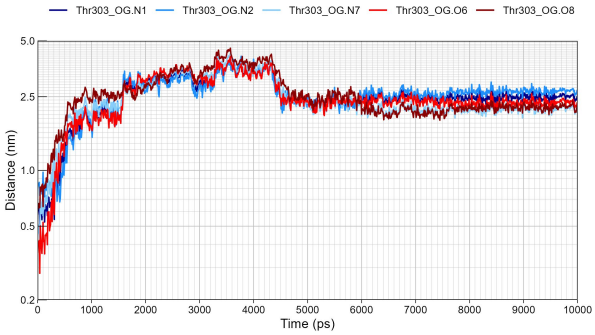
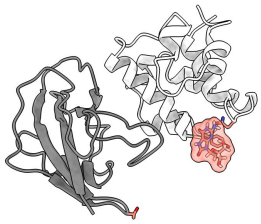
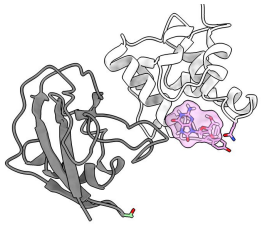
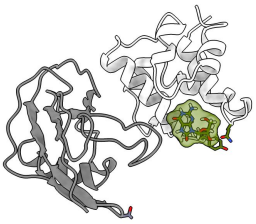
8 ns



10 ns

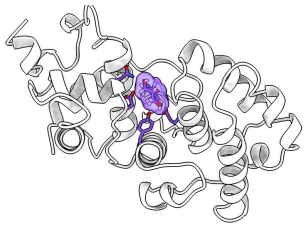
Fig S7. (C) Molecular dynamic simulation for *Marinosulfonomonas* MutY NTD complexed with adenosine. In the first 3000 ps, the adenine base approached closely catalytic Glu49 (green traces), often directly hydrogen bonded and occasionally bridged by a solvent molecule as illustrated at 2 ns. However, the complex was relatively unstable, and the ligand departed the active site and found a new binding site by 8000 ps. Favorable VDW interactions characterize both binding sites, but favorable Coulombic interactions are diminished substantially at the second site.

(D) *Marinosulfonomonas* MutY complexed with OG

0 ns		Energy 
2 ns		
4 ns		Distance OG - Thr303 
6 ns		
8 ns		**Fig S7**. (D) Molecular dynamic simulation for *Marinosulfonomonas* MutY complexed with OG. The initial ligand complex was unstable with a hinge-like motion creating new contacts between the NTD (white) and CTD (gray). After nearly escaping at ~4000 ps, the OG ligand found a more favorable binding site on the surface of the NTD.
10 ns		

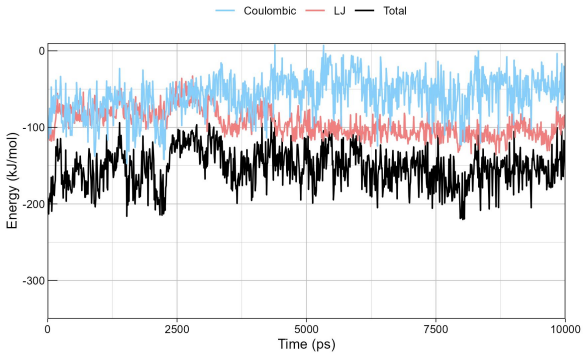
(E) *Rhodobacteraceae* MutY complexed with adenosine

0 ns



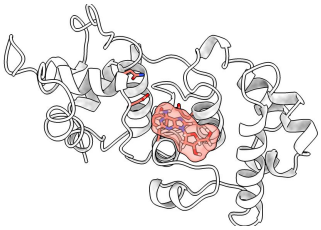
2 ns

Energy

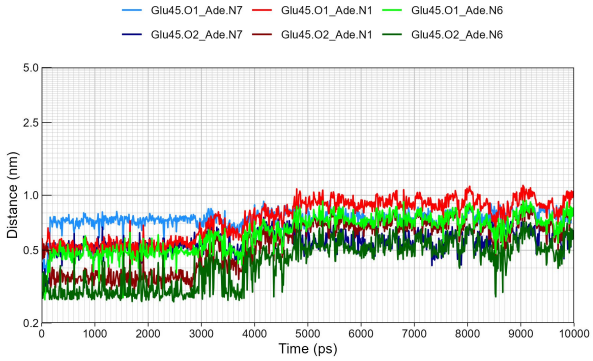


4 ns

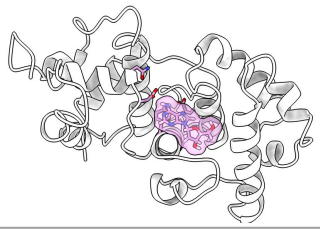
6 ns



Distance Adenosine - Glu45



8 ns



10 ns

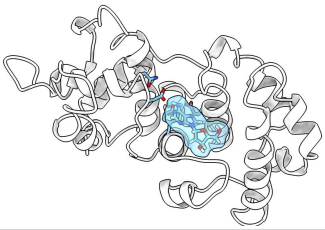
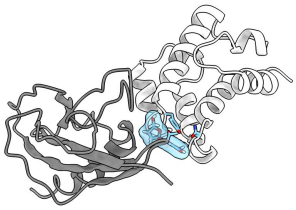


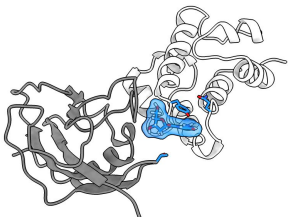
Fig S7. (E) Molecular dynamic simulation for *Rhodobacteraceae* MutY NTD complexed with adenosine. The complex was relatively unstable. The adenine base initially hydrogen bonded with catalytic Glu45 during the first 3800 ps but then changed orientation and drifted to a new site distinct and different from its original docking site.

(F) *Rhodobacteraceae* MutY complexed with OG

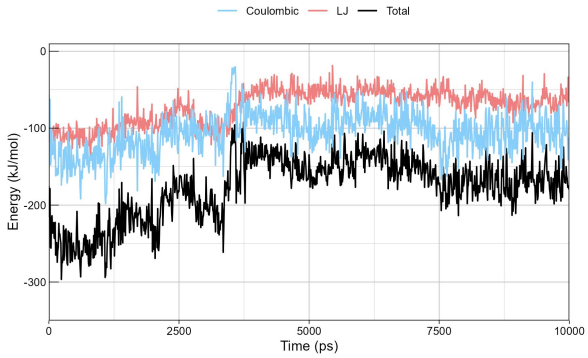
0 ns



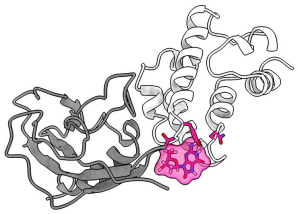
2 ns



Energy



4 ns



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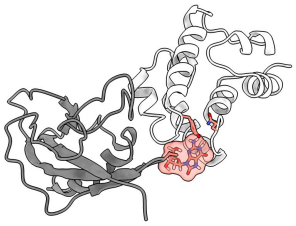
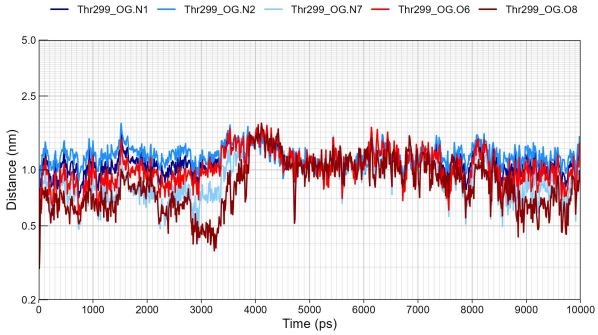


Distance OG - Thr299



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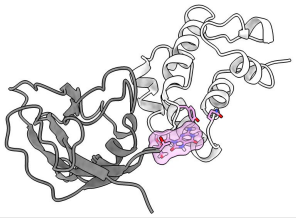


10 ns

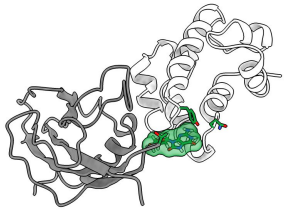
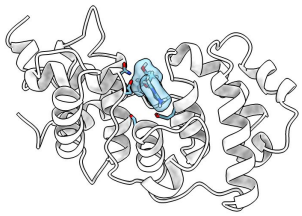


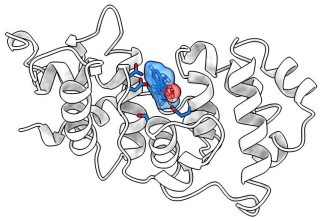
Fig S7. (F) Molecular dynamic simulation for *Rhodobacteraceae* MutY complexed with OG. The ligand complex was fairly unstable. Functionally relevant hydrogen bonds between Thr299 and OG observed for the initial starting structure at 0 ps were lost as the ligand moved to a new position closer to the NTD.

(G) *Thiotrichaceae* MutY complexed with adenosine

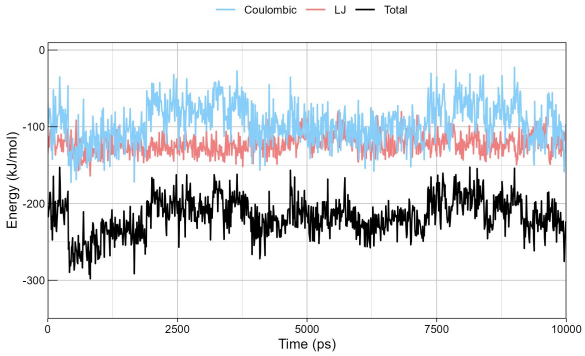
0 ns



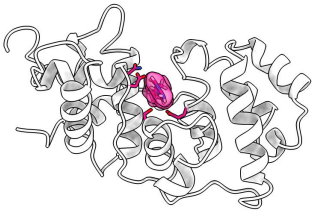
2 ns



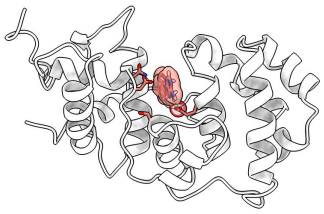
Energy



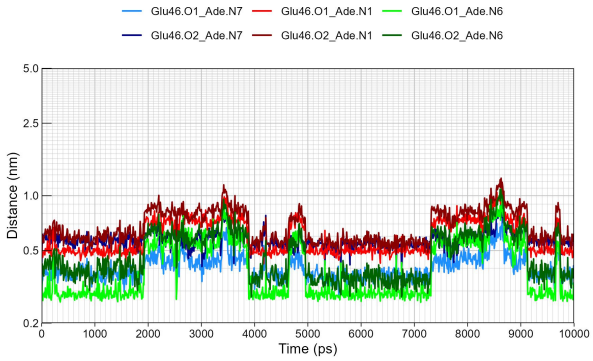
4 ns



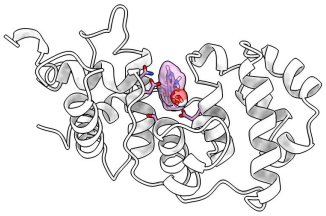
6 ns



Distance Adenosine - Glu46



8 ns

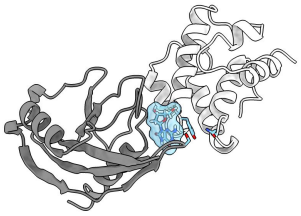


10 ns

Fig S7. (G) Molecular dynamic simulation for *Thiotrichaceae* MutY NTD complexed with adenosine. Note that Ser replaces active site Tyr for this LCHF MutY, as is also the case for *Ec* MutY. The complex was relatively stable with the ligand persisting in the active site throughout the simulation. Hydrogen bonds between catalytic Glu46 and the Hoogsteen face of adenine were evident by close distance to N7 (blue traces) and N6 (green traces) and by inspection of structures. Water frequently bridged N7 to Glu46 as seen in the structures at 2 ns, 6 ns, 8 ns, and 10 ns (surface with O red and H white).

(H) *Thiotrichaceae* MutY complexed with OG

0 ns



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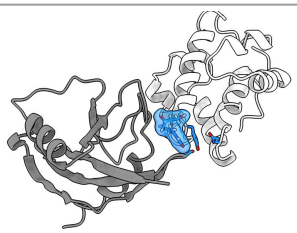

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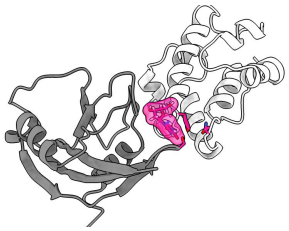

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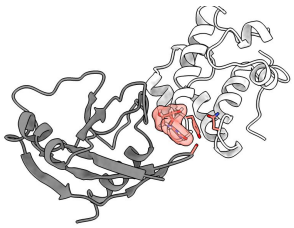

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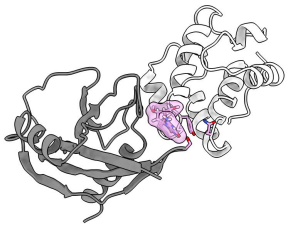

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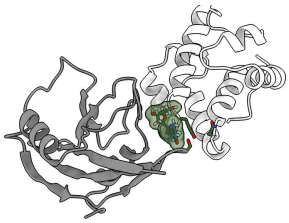

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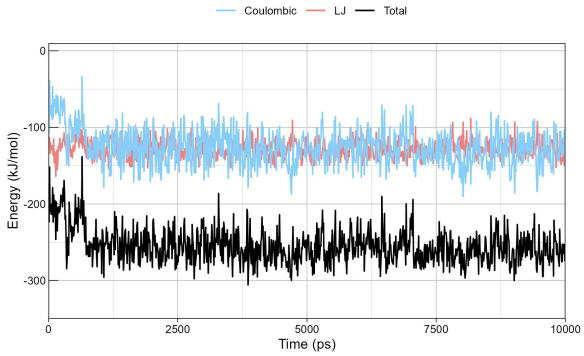


Distance OG - Ser306

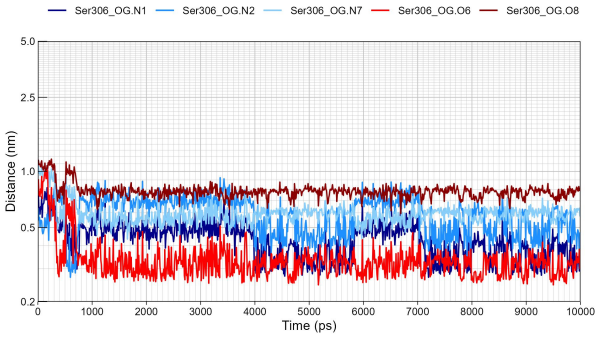
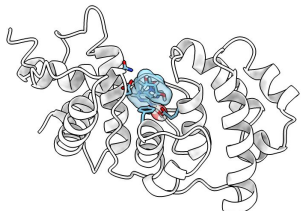


Fig S7. (H) Molecular dynamic simulation for *Thiotrichaceae* MutY complexed with OG. The ligand complex was stable for the entire 10-ns simulation with the OG ligand bound to a cleft between the NTD (white) and CTD (gray). After ~300 ps, hydrogen bonds between Ser306 and the Watson-Crick-Franklin face of OG were frequently observed as evident from close distances to O6 (red trace) and N1 (dark blue trace), and by inspection of structures.

(I) *Flavobacteriaceae* MutY complexed with adenosine

0 ns



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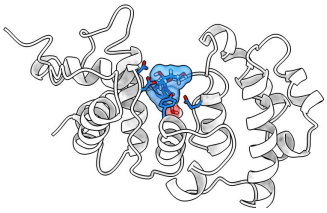

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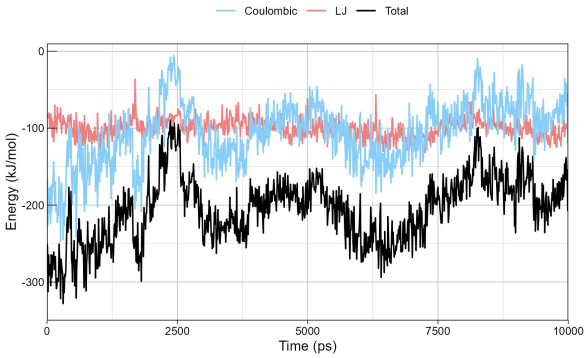

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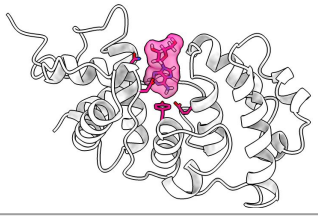

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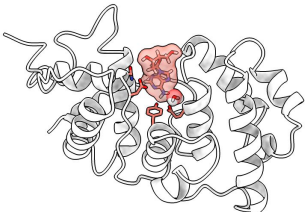
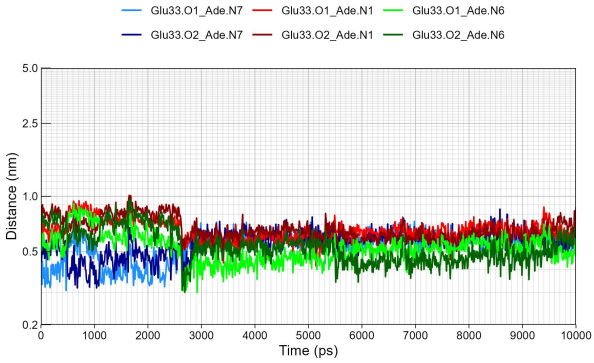


Distance Adenosine - Glu33



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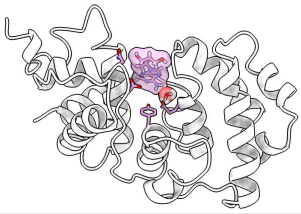

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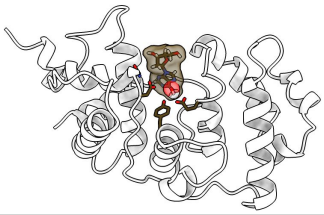
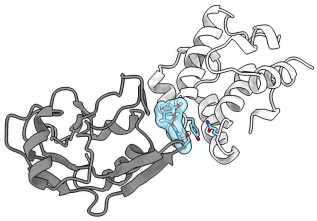


Fig S7. (I) Molecular dynamic simulation for *Flavobacteriaceae* MutY NTD complexed with adenosine. The ligand persisted in the active site throughout the simulation, with the ligand periodically finding new orientations as evident in different distance traces vying for close approach to catalytic Glu43. For example, N7 of the adenine base was very close to Glu43 (blue trace) during the first 2700 ps, suggesting catalytic engagement, but slipped out of reach at later time points. Water frequently bridged contacts between Glu43 and the adenine base (transparent surface with O red and H white).

(J) *Flavobacteriaceae* MutY complexed with OG

0 ns



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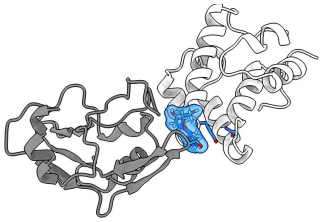

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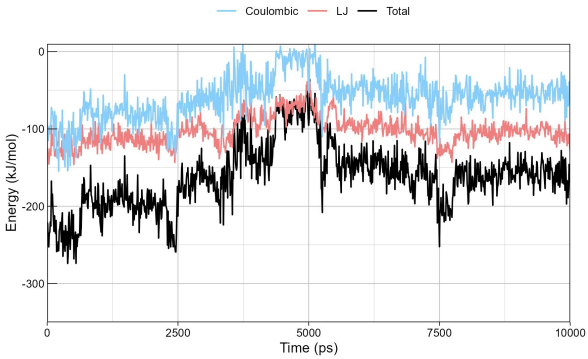

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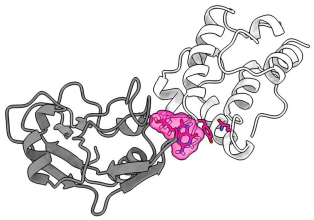

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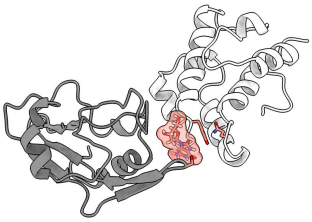
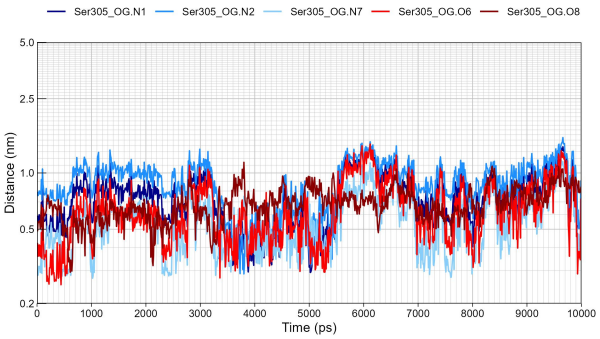


Distance OG - Ser305



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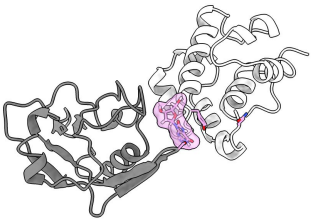

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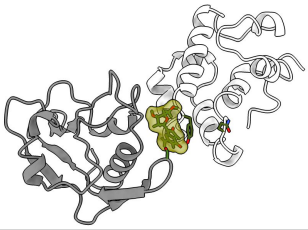

Fig S7. (J) Molecular dynamic simulation for *Flavobacteriaceae* MutY complexed with OG. The ligand complex was relatively stable yet dynamic. Functionally relevant hydrogen bonds between Ser305 and the Hoogsten face of OG can be inferred from recurring close distances and by inspection of structures.

1075 **S8 Table. Rifampicin resistance assay.**

MutY Expressed	Median (ci.low[a], ci.high[a])	Mutation Frequency[b] (per 10^8 cells)	Fold Change of EcMutY (ci.low[a], ci.high[a])	n
null	101 (81, 145)	20.26	8.78 (6.44, 13.3)	90
E. coli MutY	12 (9, 14)	6.70	1.00 (NA, NA)	79
Marinosulfonomonus MutY	7 (4, 10)	4.13	0.57 (0.27, 0.91)	30
Marinosulfonomonus MutY (catalysis-)	70 (51, 97)	30.77	6.09 (4.32, 9.30)	29
Marinosulfonomonus MutY (recognition-)	74 (59, 90)	11.53	6.39 (4.69, 8.80)	40
Rhodobacteraceae MutY	14 (8, 22)	3.48	1.22 (0.71, 2.00)	30
Rhodobacteraceae MutY (catalysis-)	61 (50, 80)	41.37	5.26 (4.00, 8.00)	38
Rhodobacteraceae MutY (recognition-)	70 (50, 82)	37.65	6.09 (4.12, 8.75)	30
Thiotrichaceae MutY	54 (42, 63)	14.77	4.70 (3.23, 6.44)	47
Thiotrichaceae MutY (catalysis-)	98 (50, 157)	28.89	8.48 (4.46, 14.28)	48
Thiotrichaceae MutY (recognition-)	72 (48, 183)	10.75	6.26 (3.77, 18.00)	20

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1077
1078 [a] Confidence intervals (95%) determined by a bootstrap method, see **Materials and methods** for details.
1079 [b] Mutation frequency reported as median number of resistant colonies per 10^8 viable colonies. Fold change was
1080 calculated by dividing Rif^R frequency by the frequency measured for cultures expressing *Ec* MutY.