Supplementary Materials

Characterizing the Specific Recognition of Xanthurenic Acid by GEP1 and GEP1-GCα Interactions in cGMP Signaling Pathway in Gametogenesis of Malaria Parasites

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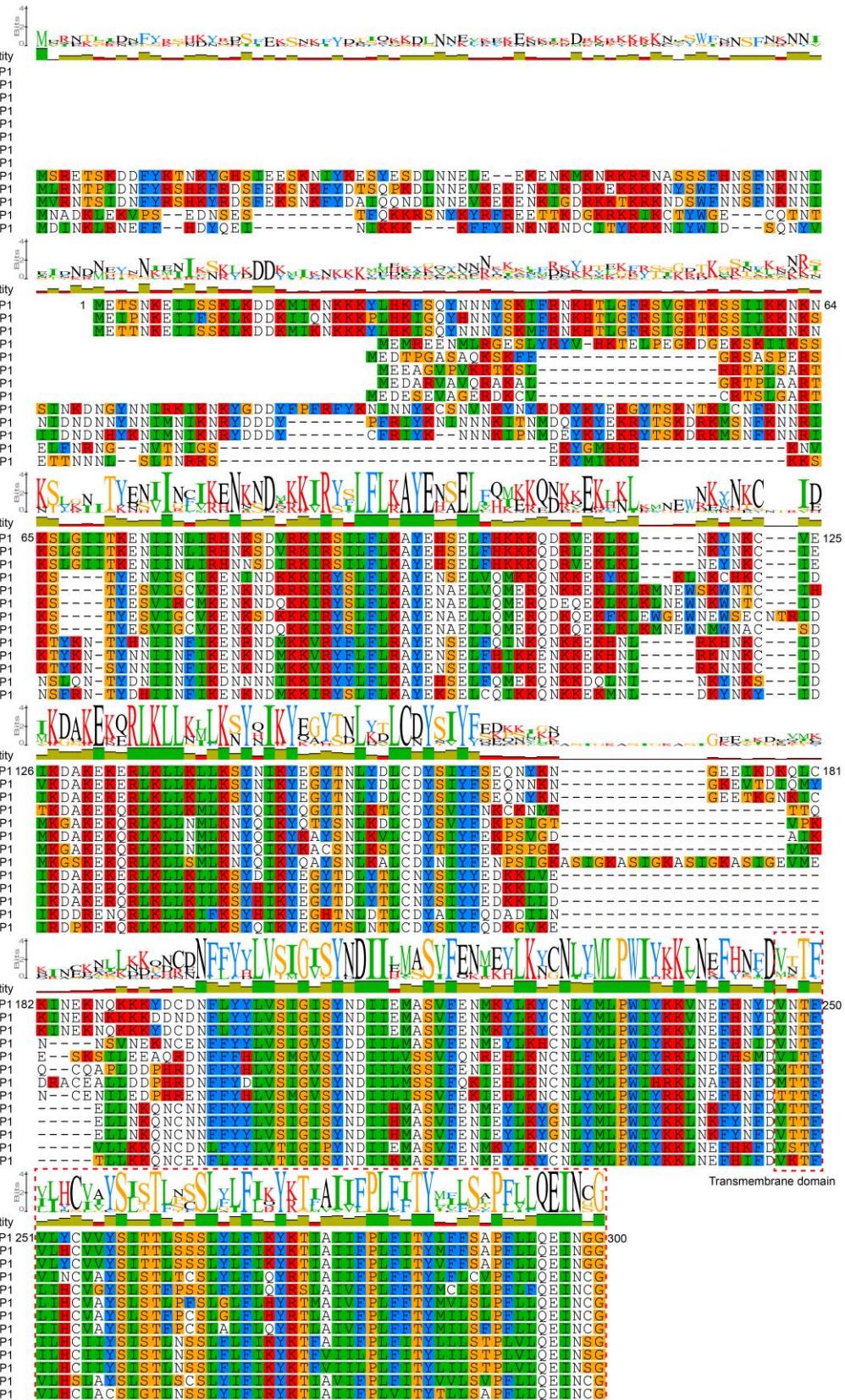
Figure S1. Multiple sequence alignment of the first 300 residues of GEP1 from 13 species of *Plasmodium*. The analysis was conducted with software geneious (http://www.geneious.com). Species used for the alignment were *Py*: *Plasmodium yelii*; *Pch*: *Plasmodium chabaudi chabaudi*; *Pbe*: *Plasmodium berghei ANKA*; *Pgo*: *Plasmodium gonderi*; *Pvi*: *Plasmodium vivax*; *Pin*:

Plasmodium inui San Antonio 1; *Pcy*: *Plasmodium cynomolgi B*; *Pkn*: *Plasmodium knowlesi strain H*; *Pfa*: *Plasmodium falciparum 3D7*; *Pga*: *Plasmodium gaboni*; *Psp*: *Plasmodium sp. gorilla clade G2*; *Pma*: *Plasmodium malariae*; *Pre*: *Plasmodium relictum*.
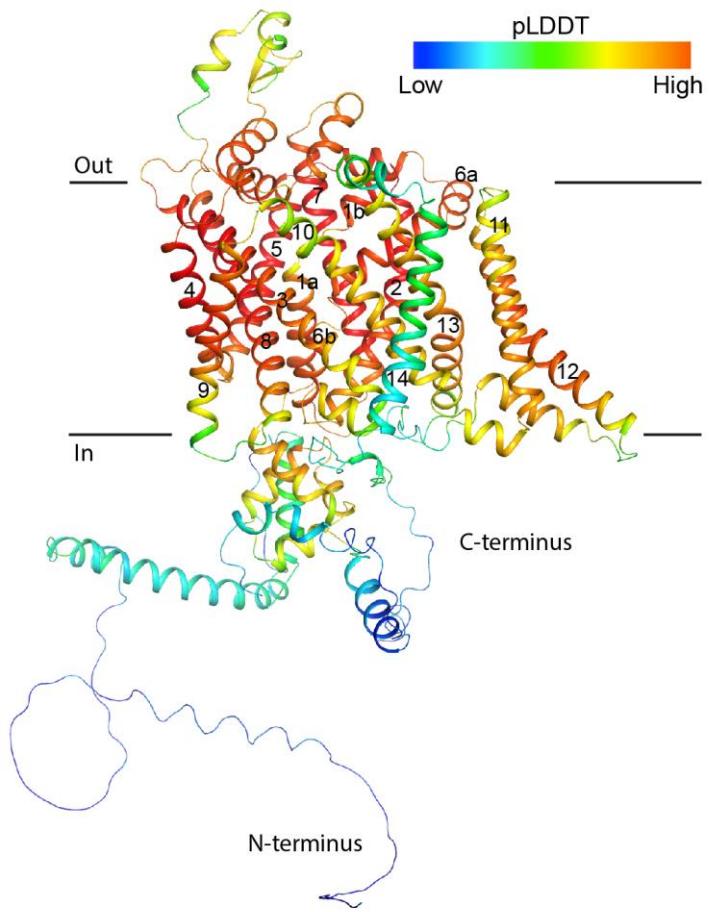
Figure S2. *De novo* structure prediction using AlphaFold2 for GEP1 protein. Model is colored by pLDDT value.
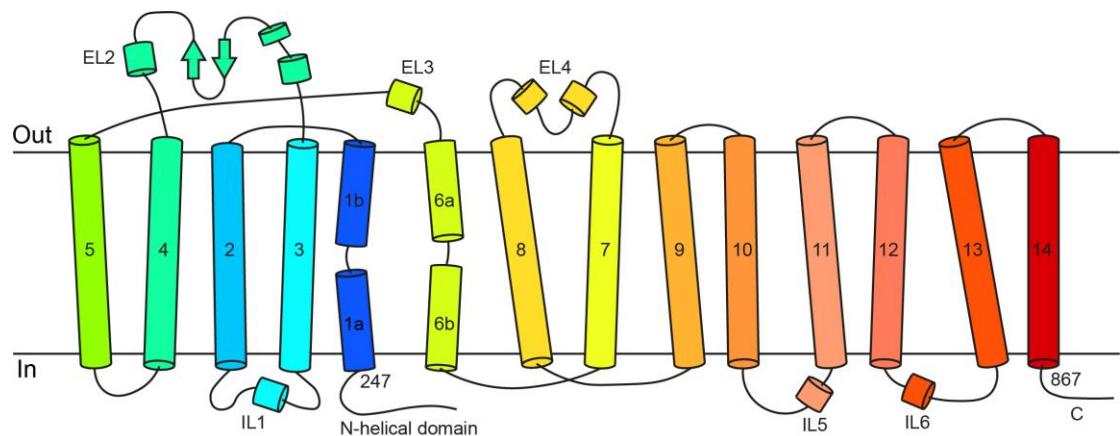
Figure S3. Membrane topology of GEP1 protein. This schematic topology representation is based on the 3D structure predicted by AlphaFold2.
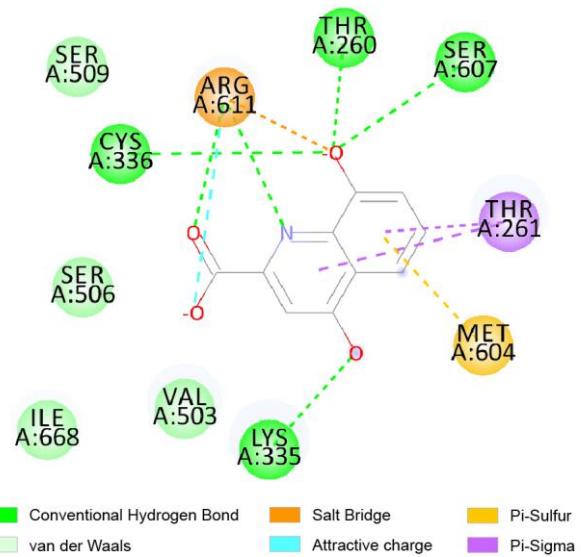
Figure S4. 2D ligand interaction diagram between XA and GEP1.
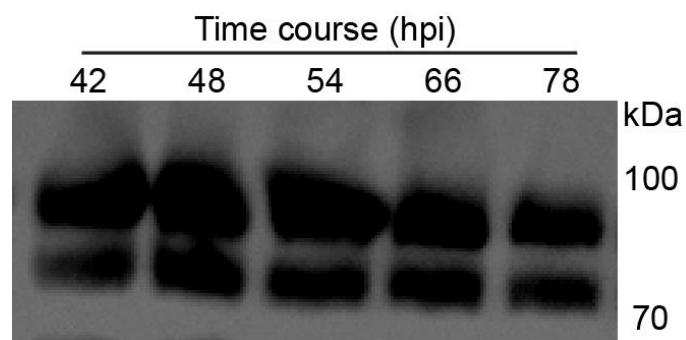
Figure S5. Comparison of expression level of mEGFP fused GEP1$^{192-905}$ with hours post-infection (hpi) by western blot, anti-rabbit GFP-tag antibody was used.
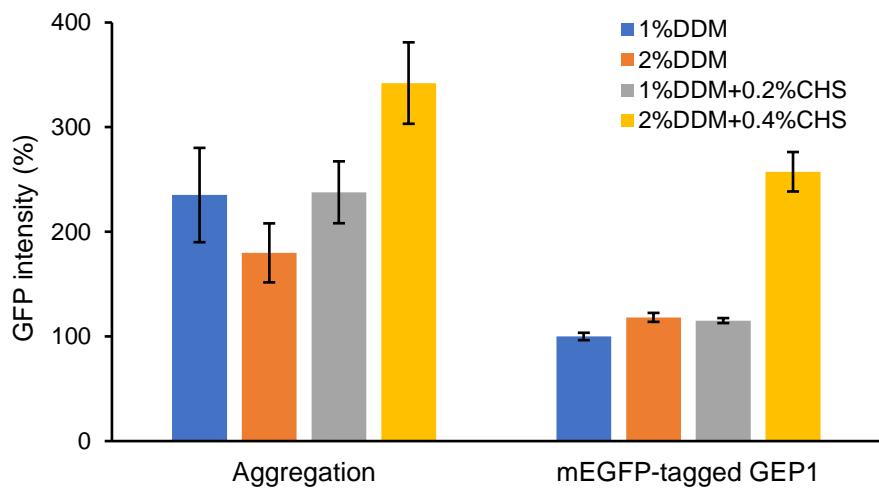
Figure S6. Optimization of detergents for the extraction of membrane protein GEP1.
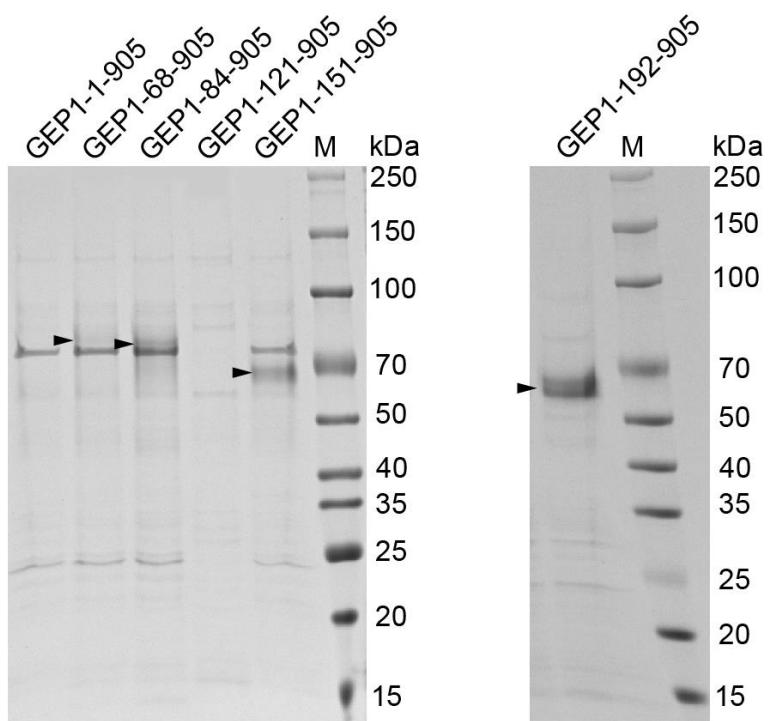
Figure S7. SDS-PAGE analysis of truncations of N-terminal GEP1 by Strep-Tactin beads. Black arrow indicated the location of target band.
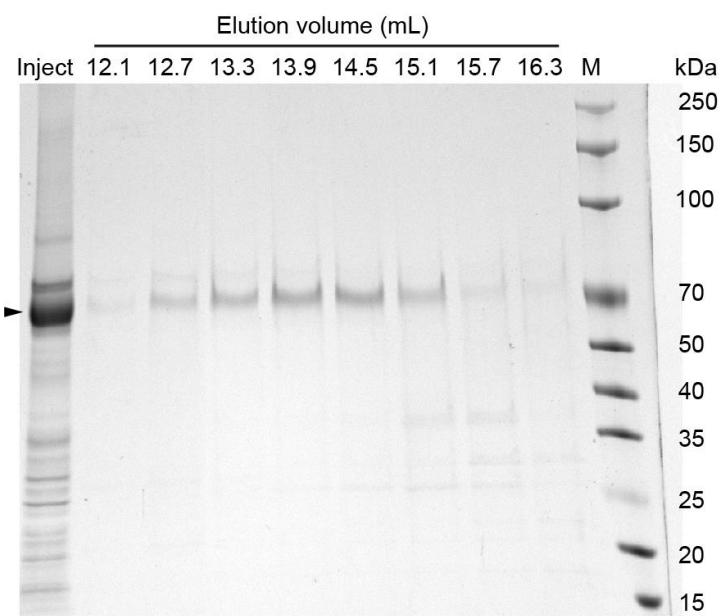
Figure S8. SDS-PAGE analysis of GEP1$^{151-905}$ purification and size-exclusion chromatography (SEC) fractions in detergent 0.03% DDM. The black triangle represented target band of GEP1$^{151-905}$.
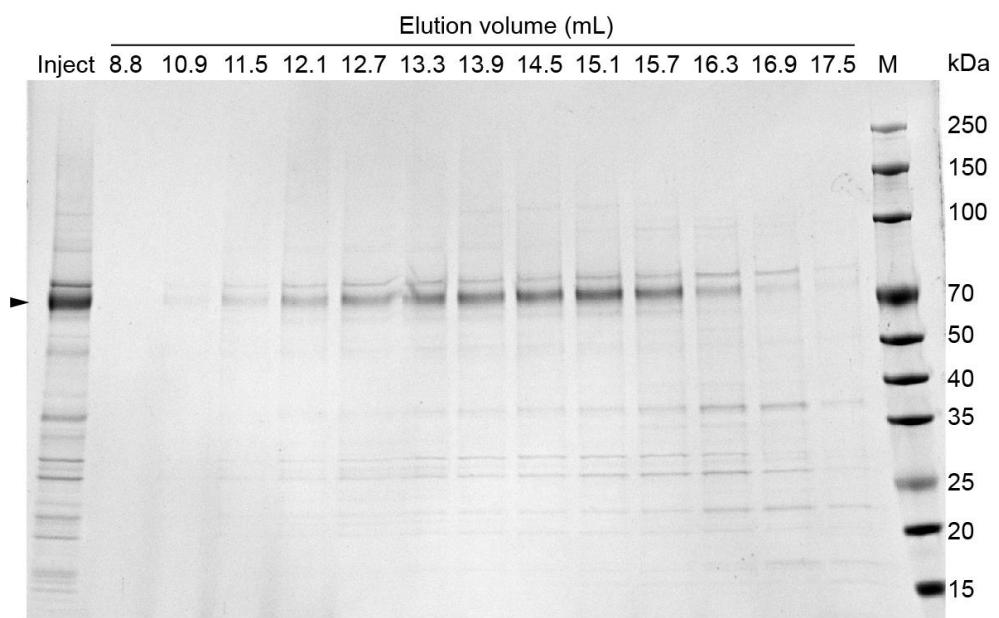
Figure S9. SDS-PAGE analysis of GEP1$^{151-905}$ purification and SEC fractions in detergent 0.001%/0.00033% LMNG/GDN. The black triangle represented target band of GEP1$^{151-905}$.

A   0.05 mg/mL

B   0.02 mg/mL
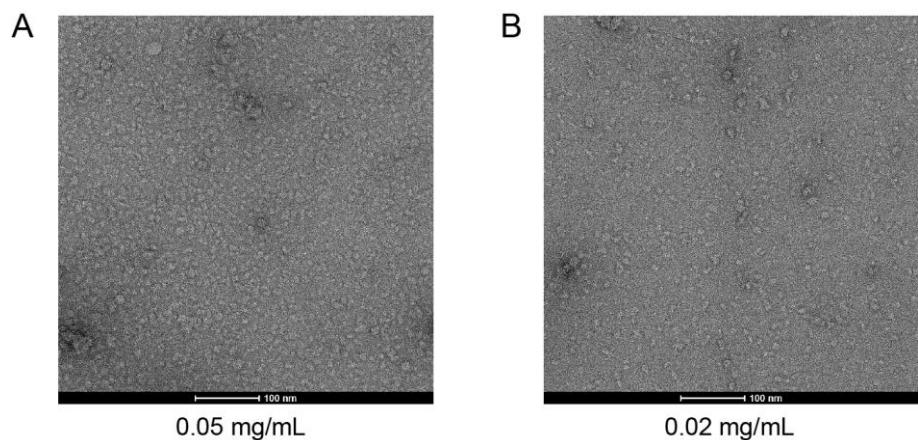
Figure S10. Negative stain electron microcopy of GEP1[151-905] in buffer (5 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.03% DDM). (A) Image of negatively stained GEP1[151-905] at concentration of 0.05 mg/mL with 2% uranyl acetate. (B) Image of negatively stained GEP1[151-905] at concentration of 0.02 mg/mL with 2% uranyl acetate.
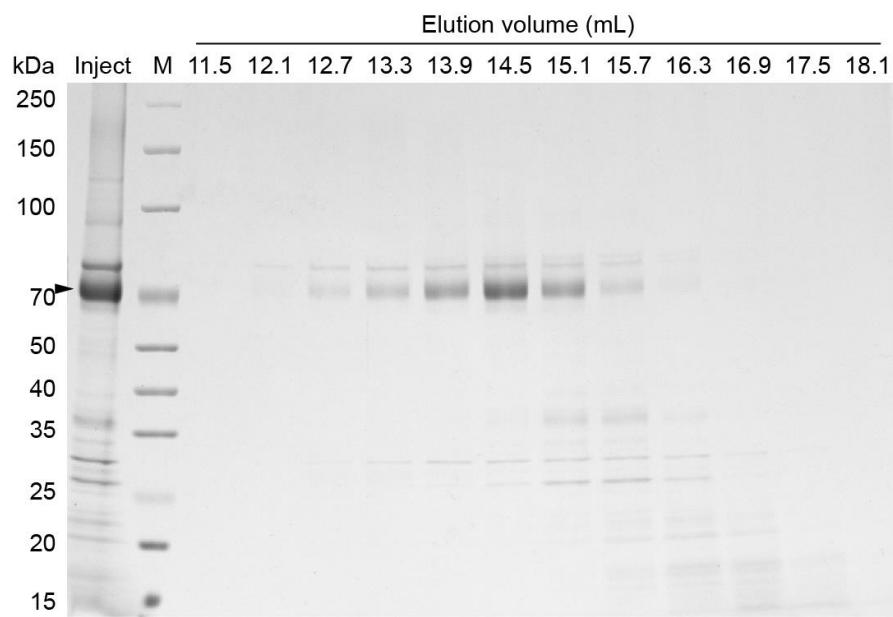
Figure S11. SDS-PAGE analysis of GEP1[151-905] purification and SEC fractions trapped in amphipol A8-35. The black triangle represented target band of GEP1[151-905].
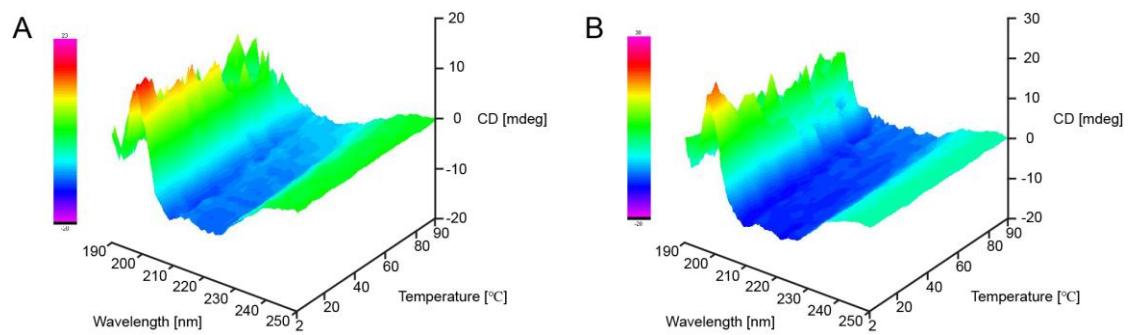
Figure S12. Three-dimensional figure of temperature-dependent CD spectra from 2°C to 92°C, protein GEP1[151-905] in buffer pH 7.4 (A) and pH 8.0 (B).
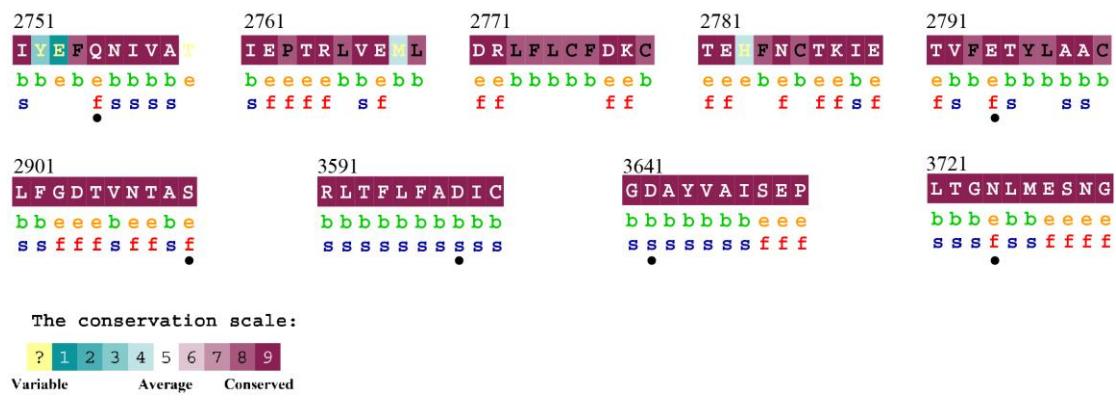
Figure S13. Conservative and functional analysis of several residues in GCα by ConSurf website (https://consurf.tau.ac.il/consurf_index.php). The more conserved residues have a darker red color, residues with low conservation tend to be cyan. Letters under the residues, e: an exposed residue according to the neural-network algorithm; b: a buried residue according to the neural-network algorithm; f: a predicted functional residue (highly conserved and exposed); s: a predicted structural residue (highly conserved and buried).

Table S1. Comparison of proteins structurally similar with GEP1 by DALI server

No.	PDB	Z-score	Description
1	7LI7	26.1	Sodium-dependent serotonin transporter
2	4MME	25.9	Transporter
3	6VRK	25.9	Sodium-dependent serotonin transporter
4	5JAG	25.8	Transporter
5	4MM4	25.5	Transporter
6	5JAE	25.5	Transporter
7	6W2B	25.4	Sodium-dependent serotonin transporter
8	6W2C	25.4	Sodium-dependent serotonin transporter
9	4MM9	25.3	Transporter
10	7LI8	25.2	Sodium-dependent serotonin transporter
11	4XPF	25.1	Dopamine transporter-protein
12	7MGW	25.0	Sodium-dependent serotonin transporter
13	4MMB	24.8	Transporter
14	4MMD	24.7	Transporter
15	5I73	24.7	Sodium-dependent serotonin transporter
16	6M3Z	24.7	Sodium-dependent serotonin transporter
17	3TT1	24.6	Leucine transporter, LeuT
18	4MMC	24.6	Transporter
19	3TT1	24.6	Leucine transporter, LeuT
20	4MMD	24.6	Transporter

Table S2. Comparison of proteins structurally similar with N-terminal helical domain of GEP1 by DALI server

No.	PDB	Z-score	Description
1	7N7S	3.3	Hydroxymethylglutaryl-CoA reductase
2	5TTE	3.1	E3 ubiquitin-protein ligase ARIH1
3	3P1W	2.9	RabGDI protein
4	7NPF	2.9	AAA family ATPase
5	7BY1	2.8	Histone acetyltransferase KAT2A
6	3FIG	2.8	2-isopropylmalate synthase
7	6IFN	2.8	Type III-A CRISPR-associated protein Csm1
8	3T6P	2.8	Baculoviral IAP repeat-containing protein 2
9	2MVT	2.7	Scoloptoxin SSD609
10	7DN9	2.7	Putative cytoplasmic protein
11	5WU1	2.6	Speckle targeted PIP5K1A-regulated poly(A) polymerase
12	4M5D	2.6	U3 small nucleolar RNA-associated protein 22
13	6HN7	2.5	Redirecting phage packaging protein C (RppC)
14	4P17	2.5	RabGAP/TBC protein
15	2PX0	2.5	Flagellar biosynthesis protein flhF
16	7SHG	2.5	Ribofuranosyl transferase
17	2KNA	2.5	Baculoviral IAP repeat-containing protein 4
18	3EZF	2.4	ParA
19	4CEJ	2.4	ATP-dependent helicase/nuclease subunit A
20	6H4C	2.4	dUTPase

Table S3. Primers used to generate constructs with different tags.

Primer no.	Primer name	Sequence (5'-3')
1	8H-GEP1-TP_Fwd	GGCGCGGATCCCGGTCCGAAGCGCATATGCATCACCATCACCATC
2	8H-GEP1-TP_Rev	CGTCGACGTAGGCCTTTGAATTCCGCTCAGCCTCTGATGGAAAACTCG
3	8H-GEP1-mEGFP-TP_Fwd	CGGCGAGTTTTCCATCAGAGGCCTGGAAGTTCTGTTCCAGG
4	8H-GEP1-mEGFP-TP_Rev	CGACGTAGGCCTTTGAATTCCGCTCACTTGTACAGCTCGTC
5	8H-mEGFP-GEP1-TP_Fwd	CACCATCATCACCACGGATCCATGGTGAGCAAGGGCGAGG
6	8H-mEGFP-GEP1-TP_Rev	GCCCCTGGAACAGAACTTCCAGGCCGCTCTTGTACAGCTCG
7	HA-GEP1-GEP1-TP_Fwd	CGGTCCGAAGCGCATATGAAGACGATCATCGCCCTGAGCTACATCTTC
8	HA-GEP1-GEP1-TP_Rev	GTCTCCGGCCCGGATCCGGCGAATACCAGGCAGAAGATGTAGCTCAGG
9	ME-GEP1-GEP1-TP_Fwd	GGTCCGAAGCGCATATGAAATTCTTAGTCAACGTTGCCCTTGTTTTTATGGTCGTAT
10	ME-GEP1-GEP1-TP_Rev	CTGGTCTCCGGCCCGGATCCATCCGCATAGATGTAAGAAATGTATACGACCATAAAAAC

Table S4. Primers to generate N-terminal truncations of GEP1.

Primer no.	Primer name	Sequence (5'-3')
11	GEP1-50-905_Fwd	CATATGGGATCCGGGCCGAGCGTGGGCAGAACCAAG
12	GEP1-50-905-Rev	CTTGGTTCTGCCCACGCTCGGCCCGGATCCCATATG
13	GEP1-68-905_Fwd	GGTCCGAAGCGCATATGGGCATCATCACCAAGGAG
14	GEP1-68-905_Rev	CTCCTTGGTGATGATGCCCATATGCGCTTCGGACC
15	GEP1-84-905_Fwd	GGTCCGAAGCGCATATGAGCGACGTGAGGAAGATTAG
16	GEP1-84-905_Rev	CTAATCTTCCTCACGTCGCTCATATGCGCTTCGGACC
17	GEP1-121-905_Fwd	GGTCCGAAGCGCATATGAACAAGTGTGTCGAGATTAAG
18	GEP1-121-905_Rev	CTTAATCTCGACACACTTGTTCATATGCGCTTCGGACC
19	GEP1-151-905_Fwd	CATATGGGATCCGGGCCGTACACCAACCTCTACGAC
20	GEP1-151-905-Rev	GTCGTAGAGGTTGGTGTACGGCCCGGATCCCATATG
21	GEP1-192-905_Fwd	CATATGGGATCCGGGCCGTACGATTGCGACAACTTTC
22	GEP1-192-905-Rev	GAAAGTTGTCGCAATCGTACGGCCCGGATCCCATATG

Table S5. Primers to generate mutations of GCα-C.

Primer no.	Primer name	Sequence (5'-3')
23	Q2755A_Fwd	GTGATATTTATGAATTTGCAAATATAGTTGCAACTATTG
24	Q2755A-Rev	CAATAGTTGCAACTATATTTGCAAATTCATAAATATCAC
25	E2794A_Fwd	GAAACAGTTTTTGCAACATATTTAGCTGCTTG
26	E2794A_Rev	CAAGCAGCTAAATATGTTGCAAAAACTGTTTC
27	S2910W_Fwd	GATACGGTTAATACTGCTTGGCGAATGAAAACAACTGG
28	S2910W_Rev	CCAGTTGTTTTCATTCGCCAAGCAGTATTAACCGTATC
29	D3598A_Fwd	CATTCTTATTTGCTGCAATATGTGGATTTACTTC
30	D3598A_Rev	GAAGTAAATCCACATATTGCAGCAAATAAGAATG
31	D3642V_Fwd	TAAATTATGTACAATTGGAGTAGCATATGTTGCAATAAG
32	D3642V_Rev	CTTATTGCAACATATGCTACTCCAATTGTACATAATTTA
33	N3724W_Fwd	GATGTATTAACTGGTTTCCTTATGGAAAGTAATGG
34	N3724W-Rev	CCATTACTTTCCATAAGGAAACCAGTTAATACATC