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			PTAR	1 N-terminal don	nain		
		a1		β1		β2	β3
Human	1	MAENSEEVAVI.VORVVK	DITNAFRRNP	IDETGUTEC	PEARYNRSPI	VLVENK	LGVESWC
Cow	1	MAETSEEVAVLVÕRVVK	DITNAFRRNP	HIDEIGLIPC	PEARYNRSPI	VLVENK	LGVESWC
Mouse	1	MAE <mark>sn</mark> eevavlvõrvvki	DITNAFRRNP	HIDEIGLIPC	PEARYNRSPI	VLVENK	LGVESWC
Clawed frog	1	MAEWKEEVEVLVQRVVKI	DIT <mark>G</mark> AF <u>R</u> RNP	NIDEIGLIPC	PEATYNRSPI	VLVENK	LGVESWC
Zebrafish	1	MAESEEEVDVLVQRVVK	DITNAF <mark>K</mark> RNP	NIDEIGLIPC	PEARYNRSPI	VLVENK	LGVESWC
Fruit fly	1	MEEERNEKKVLCEKIIR	DINAVELKDQI	DLASFEIIPK	– EANCNKSPV	VHVEHN	LGLESWC
		a2			α3		α4
Human	61	VKFLLPYVHNKLLLYRT	R	KQWLNRDELI	DVTCTLLLLN	PDFTTA	WNVRKEL
Cow	61	VKFLLPYVHNKLLLYRT	R	KQWLN <mark>K</mark> DEL <u>I</u>	DVTCTLLLLN	PDFTTA	WNVRKEL
Mouse	61	VKFLLPYVHNKLLLYRT	3	KQWLNKDELA	DVTCTLLLLN	PDFTTA	WNVRKEL
Clawed frog	61	IKFLLPYVHNKLLLYRO	K	KUWLNRDELI	DVTCTLLLLN	PDFTTA	WNVRKEL
Zebransn Fruit fly	60	AOHWYDHAHRTLISHRR			KVI.NVALLIN	PDFTTA	WHTRROT.
Truit ity			2				
Humon		d5		do			
Cow	112		ALTKFPKSPE:	LMIHKKMAP	QLIQETSLPS OUTOBECLPS	FVTKG-	NLGTIPT
Mouse	112	TISGTLSPIKDLHLGKL	ALIKFPKSPE: Altrkfpkspe:	TWIHRRWVLQ	OLSOFTELPS	SWAKG-	SLCAVDA
Clawed frog	112	IOSGTLNPWKDLOLGKL	ALTKFPKSPE'	TWIHRRWVLO	RVVOFLVVAA	VVGKD-	ATCPETY
Zebrafish	112	LOCGVLNPEKDLYLGKL	ALSKUPKSPE	ſWIHRRWVLQ	RLQKECSPSC	QELKDS	AESRRQC
Fruit fly	120	VQKNRLSINKELQFSAL	<b>VLSIKPKS</b> NE	AFAY <mark>RRW</mark> LYS	FQSADA		
		α7		a8		α9	
Human	171	ERAORITOEEMEVCGEA	AGRYPSNYNA	WSHRIWVLOH		DELSST	KHWASMH
Cow	171	ERTOOLIREEMEVCGEA	AGRYPSNYNA	WSHRIWVLOH	LAKLDTKILI	DELSST	KHWASMH
Mouse	171	ERTÕRIIQEEMEVCSEA	AGRYPSNYNA	WSHRIWVLÕN	VAKLD <mark>L</mark> KILI	DELSST	KHWASMH
Clawed frog	171	ERIQTIVQEEMHVCYEA	AGRYPSNYN <mark>S</mark>	WSHRIWV <mark>I</mark> QH	LGNLNVKLLI	DELSST	<u>кнw</u> vsмн
Zebrafish	172	ERLORALQEEMRVCAEA	AGRYPSNYNA	WSHRIWVLQN	M <mark>AK</mark> GNL <u>K</u> VLH	DELSST	RLWVSMH
Fruit fly	163	IDWPNDIGIGERAT	ADRCASNYHA	WSHRQWILQN	GPC <b>L<u>u</u>Ç</b>	SDULRI	EKFMRK
		a10					
Human	231	VSDHSGFHYRQFLLKSL:	ISQTVID	SSVMEQNPLR	SBPAI	VPPKD-	EEAAVST
Cow	231	VSDHSGFHYRQFLLKSL	ISQTVTD	GSVLEQSPLR	S <sup>D</sup> STV	VLPKD-	EEAVASA
Mouse	231	VSDHSGFHYRQFLLKSL	ISQTTID	SAVPQHNSKK	<u>S</u> <u>B</u>	<u>PKD</u> -	EAAAAST
Zebrafieh	231	VSDHSGFHYRQFLLKSL		SDNVTAVEDL	IANER	NPCLP-	REGEAIW
Fruit fly	215	TSDVSCVHVROVI I SRA	VELSFALPK	SGASGSSTIA	 St.oht.mtsv6	LECEAN	
,	210			a11			 ว
Human	202						
Cow	282			-LLEEEVEFS	TDLIDSIPGE	ETLWCH	RRHVFVI.
Mouse	277	EEPSVNLPO		LLEEEVEFC	TDLIDSYPGE	ETLWCH	RRHVFYL
Clawed frog	282	NQICFDLPY		- III DEEMKLN	RELLDSYPGH	ETLWCH	RRQIFKL
Zebrafish	278	NHHKDVMPR		-LFHDEIQLC	TDLIESYPGH	ETLWCH	RRHVFYL
Fruit fly	270	DLLGLLDHVDLSSVSK	QRLISFLYCC	NVAANDMRLC	AEQRLMYGSF	DCFEL	RRAALKF
Human	323	QHHLNGRFPHSMTQL	SPADSPG	GTLS-DLHLI	PAGSQLSQAM	IEVDGLN	DSSKQ-G
Cow	323	QHHLNGRFPHSVIQL	SPAD <mark>S</mark> PG	GTLS-DLHGI	PAGPHTSQAM	EVDGLS	DFSKQ-G
Mouse	318	<u>OHHLNGRLPPNL</u> THL	SPADCPG	GALN-DSLQI	PTSPQLSQAM	IEVDGLS	DSSKQ-G
Zobrafich	323	THOULLEOSOSA HPO	STSASITD	C SGNISHLSS	TFQSYVINP	DVDGMS	DPNKO-G
Fruit fly	330	TVEOCVELONGLENC	LPVASGDLRS	VLUBKEL HIGNFDF	EDNEFISAVE	REES	
	555	TITE COMPENSATING					1100
Human	376			OVI.STCRNVE		WI.VTIS	0_
Cow	376	YSOENKRLKRTPAPDSL	GLEMEHRFID	OVLSTCRNVE	OARFANAYRK	WLVTLS	<u>0</u> _
Mouse	371	YSOETKRLKRTPAPDSL	GLEMEHRFID	OVLSTCRNVE	<b>ÖVRYANAYRK</b>	WLVTLS	õ-
Clawed frog	378	YTQETKRLKRAPVODSL	SLDSELRFIN	VLTNCCSPE	<b>OSRFAASYRK</b>	WLLSLO	GY
Zebrafish	372	<b>YTQDTKRLKR</b> GPLLLQP	GFPSEHTFISI	RILTGCRNPE	<b>QSRFAIAYR</b> K	WLDSVĨ	GQ
Fruit fly	382	KHRRWCNLHLSF	YPAD				

## Appendix Figure S1. Sequence alignment of PTAR1 orthologues.

Amino acid sequence alignment of PTAR1 orthologues using Clustal Omega. Identical residues are shaded in black and similar residues are shaded in gray. The secondary structure elements of the human PTAR1 (residues 1–327) are shown above the alignment. Residues whose side chain is involved in the interaction with Ykt6 are labeled with an orange circle (site 1) and a green circle (site 2). Human, Homo sapiens XP\_005252033; Cow, Bos taurus XP\_005210075; Mouse, Mus musculus NP\_082484; Clawed frog, Xenopus laevis NP\_001106898; Zebrafish, Danio rerio NP\_001123546; Fruit fly, Drosophila melanogaster NP\_569992.



## Appendix Figure S2. Purification and analysis of GGTase-III and Ykt6 mutants.

- A Geranylgeranylation activity of truncated GGTase-III (PTAR1 residues 1–327) purified from E. coli (100 nM) and full-length GGTase-III purified from Sf9 insect cells (100 nM). Cys195-farnesyl Ykt6<sup>ΔAIM</sup> (5 μM) and <sup>3</sup>H-GGPP (1 μM) were used as substrates.
- B SDS-PAGE and Coomassie staining analysis of purified GGTase-III mutants consisting of mutant PTAR1 and WT RabGGTβ.
- C SDS-PAGE and Coomassie staining analysis of purified Ykt6 mutants.
- D SDS-PAGE and Coomassie staining analysis of purified GGTase-III mutants consisting of WT PTAR1 and the G49L or G49I mutant of RabGGTβ.
- E Left, SDS-PAGE and Coomassie staining analysis of purified untagged Ykt6, untagged Ykt6 F42E mutant, and N-terminally His6-tagged Ykt6. Right, geranylgeranylation of untagged Ykt6, His6-Ykt6, and untagged Ykt6 F42E mutant by GGTase-III. Ykt6 proteins (5 μM) were incubated with or without GGTase-III (100 nM) and <sup>3</sup>H-GGPP (1 μM) for 30 min at 37°C, and the amount of 3H-geranylgeranyl transferred to Ykt6 was quantified by scintillation counting (mean ± SEM, *n* = 3).

Molecule name	Apo-GGTase-III	GGTase-III–Ykt6	GGTase-III–Cys195- farnesyl Ykt6–GGPP	GGTase-III–Cys194- geranylgeranyl, Cys195-farnesyl Ykt6 <sup>AAIM</sup> –PPi
PDB ID	6J6X	6J74	6J7X	6J7F
Data collection				
Beamline	SPring-8 BL41XU	SPring-8 BL41XU	SPring-8 BL41XU	SPring-8 BL41XU
Space group	<i>P</i> 6 <sub>5</sub> 22	P41212	P41212	P41212
Cell constants				
a, b, c (Å) α, β, γ (°)	88.5, 88.5, 647.6 90, 90, 120	119.2, 119.2, 212.6 90, 90, 90	119.2, 119.2, 211.0 90, 90, 90	119.4, 119.4, 210.7 90, 90, 90
Resolution (Å)	50–2.96 (3.01–2.96)	50–3.21 (3.27–3.21)	50–2.75 (2.80–2.75)	50–2.88 (2.93–2.88)
R <sub>sym</sub>	0.204 (1.848)	0.144 (0.490)	0.114 (0.678)	0.136 (0.512)
CC <sub>1/2</sub>	(0.578)	(0.531)	(0.886)	(0.515)
Ι/σΙ	29.0 (1.7)	12.2 (2.8)	30.7 (1.5)	12.3 (2.1)
Redundancy	69.8 (59.5)	15.5 (9.5)	21.2 (11.3)	16.2 (9.8)
Completeness (%)	100 (100)	99.7 (99.7)	98.1 (87.5)	99.9 (99.9)
Refinement				
Resolution (Å)	49.5–2.96	48.5–3.20	48.2–2.75	48.2–2.88
No. reflections	32,986	25,689	39,494	35,058
Rwork/Rfree	0.266/0.298	0.237/0.285	0.211/0.251	0.206/0.249
No. atoms				
Protein	4,672	6,417	6,398	6,514
Ligand/ion	9	_	32	19
B-factors (Å <sup>2</sup> )				
Protein	95.5	81.0	89.9	66.6
Ligand/ion	90.1	-	78.8	92.1
Rmsds				
Bond lengths (Å)	0.002	0.002	0.002	0.002
Bond angles (°)	0.516	0.461	0.521	0.500
Ramachandran plot				
Favored (%)	96.4	95.0	96.4	97.0
Outliers (%)	0.0	0.0	0.0	0.0

## Appendix Table S1. Data collection and refinement statistics.

Highest-resolution shell is in parentheses.