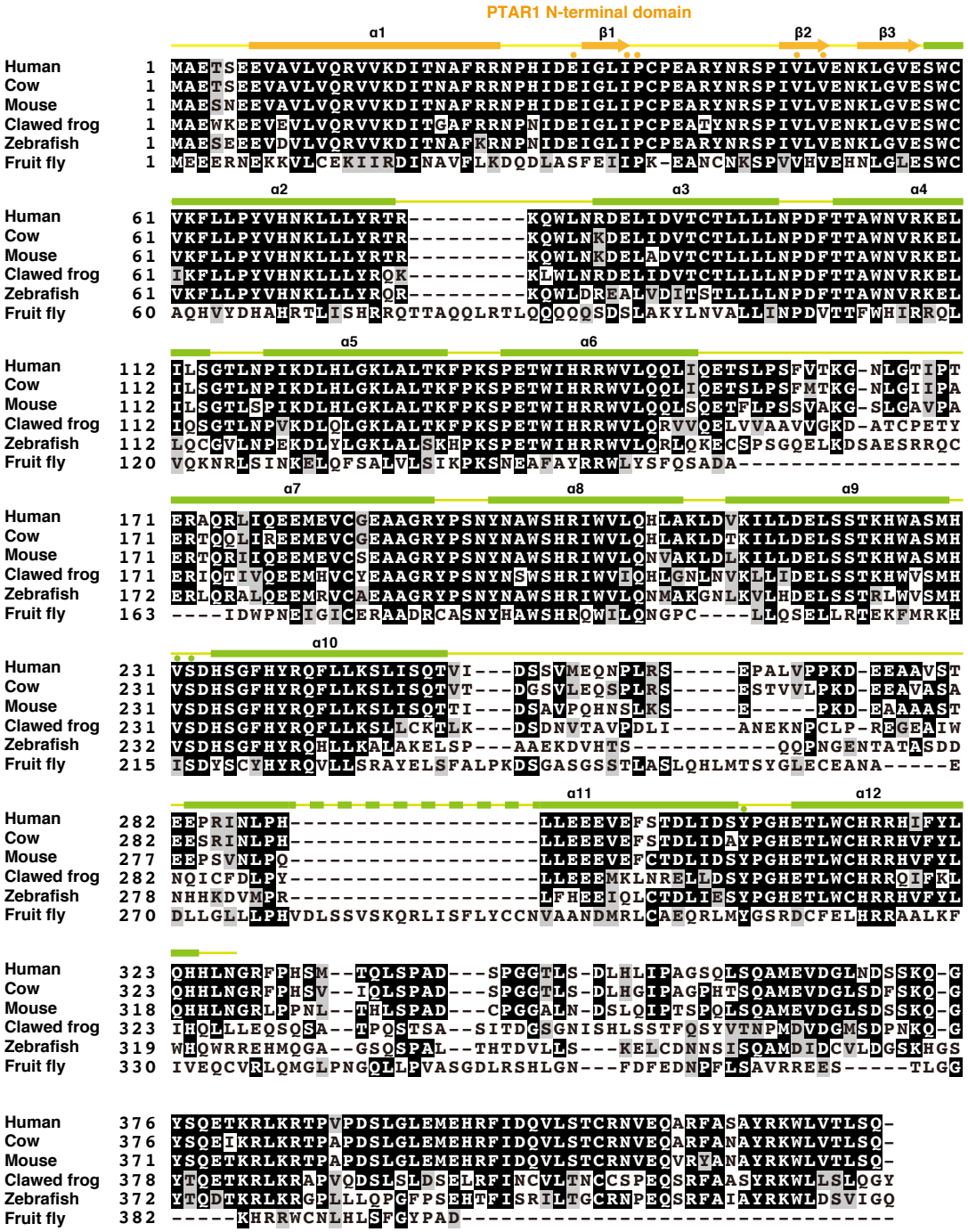
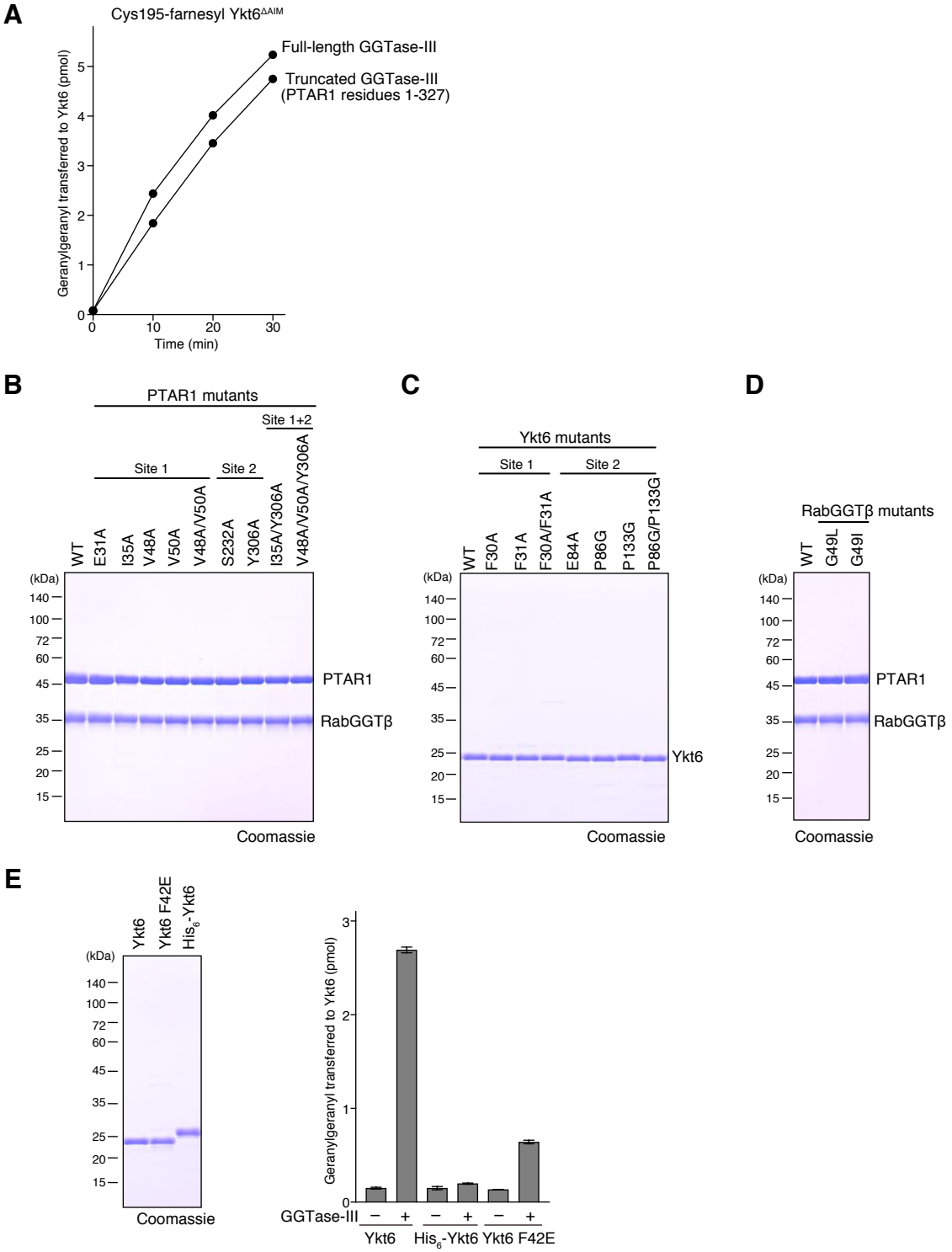
Appendix

Contents:



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$^{\Delta AIM}$ (5 μM) and ^{3}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 ^{3}H-GGPP (1 μM) for 30 min at 37ºC, and the amount of 3H-geranylgeranyl transferred to Ykt6 was quantified by scintillation counting (mean ± SEM, $n = 3$).

Appendix Table S1. Data collection and refinement statistics.

Molecule name	Apo-GGTase-III	GGTase-III–Ykt6	GGTase-III–Cys195-farnesyl Ykt6–GGPP	GGTase-III–Cys194-geranylgeranyl, Cys195-farnesyl Ykt6$^{\Delta AIM}$–PPi
PDB ID	6J6X	6J74	6J7X	6J7F
Data collection				
Beamline	SPring-8 BL41XU	SPring-8 BL41XU	SPring-8 BL41XU	SPring-8 BL41XU
Space group	$P6_522$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell constants				
a, *b*, *c* (Å)	88.5, 88.5, 647.6	119.2, 119.2, 212.6	119.2, 119.2, 211.0	119.4, 119.4, 210.7
α, *β*, *γ* (°)	90, 90, 120	90, 90, 90	90, 90, 90	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_{sym}	0.204 (1.848)	0.144 (0.490)	0.114 (0.678)	0.136 (0.512)
$CC_{1/2}$	(0.578)	(0.531)	(0.886)	(0.515)
$I/\sigma I$	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
R_{work}/R_{free}	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^2)				
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

Highest-resolution shell is in parentheses.