Supplementary Information for

The cytoplasmic synthesis and coupled membrane translocation of eukaryotic polyphosphate by signal-activated VTC complex

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[#]These authors contributed equally to this work.

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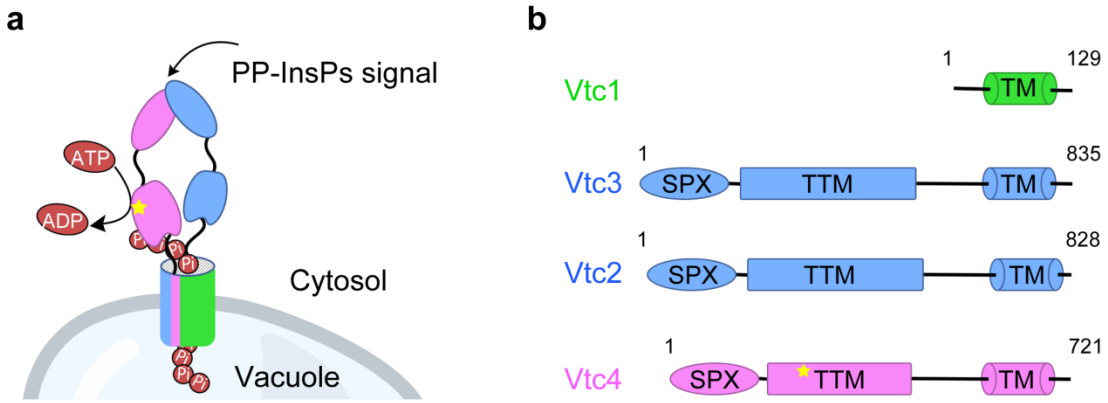
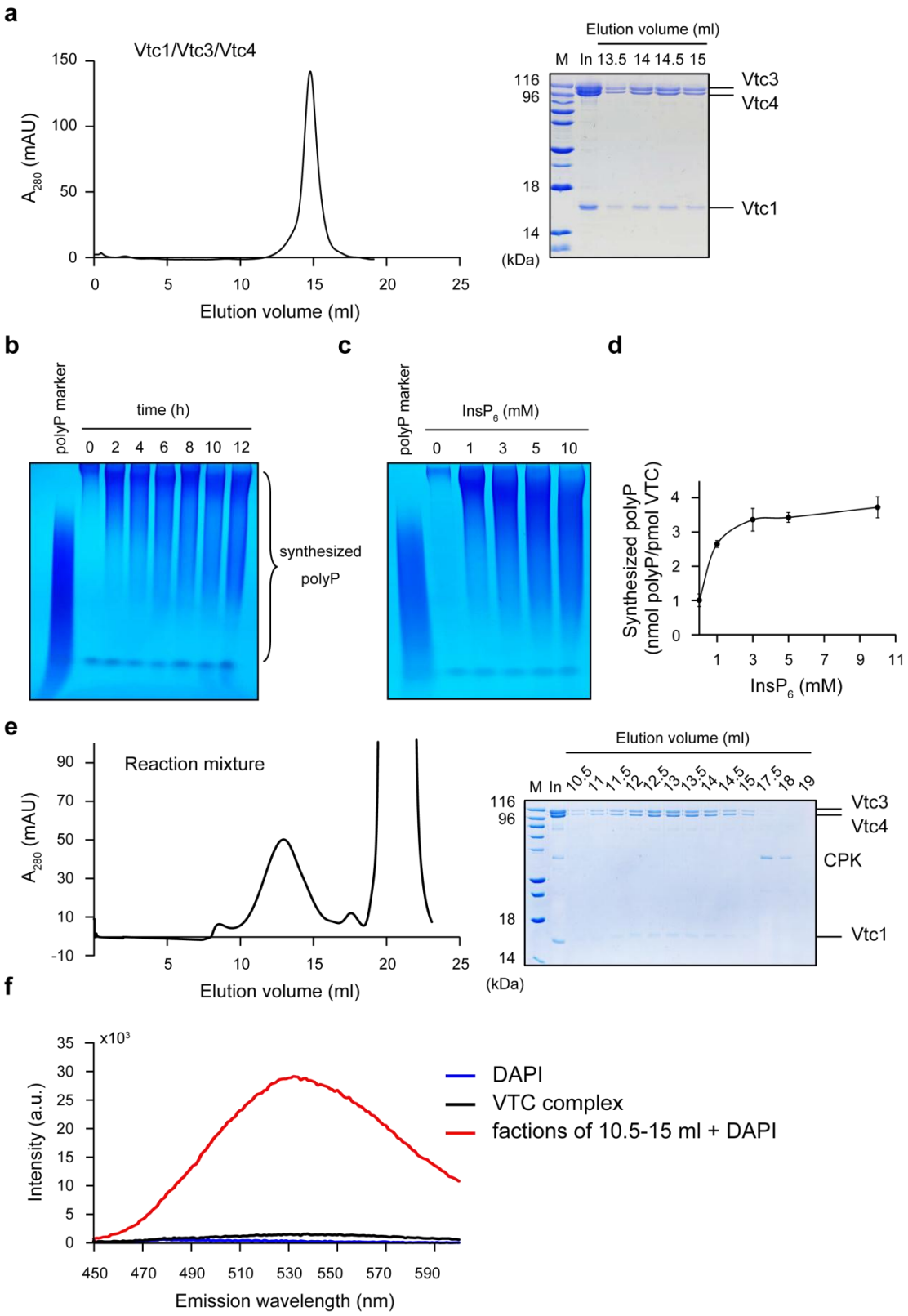
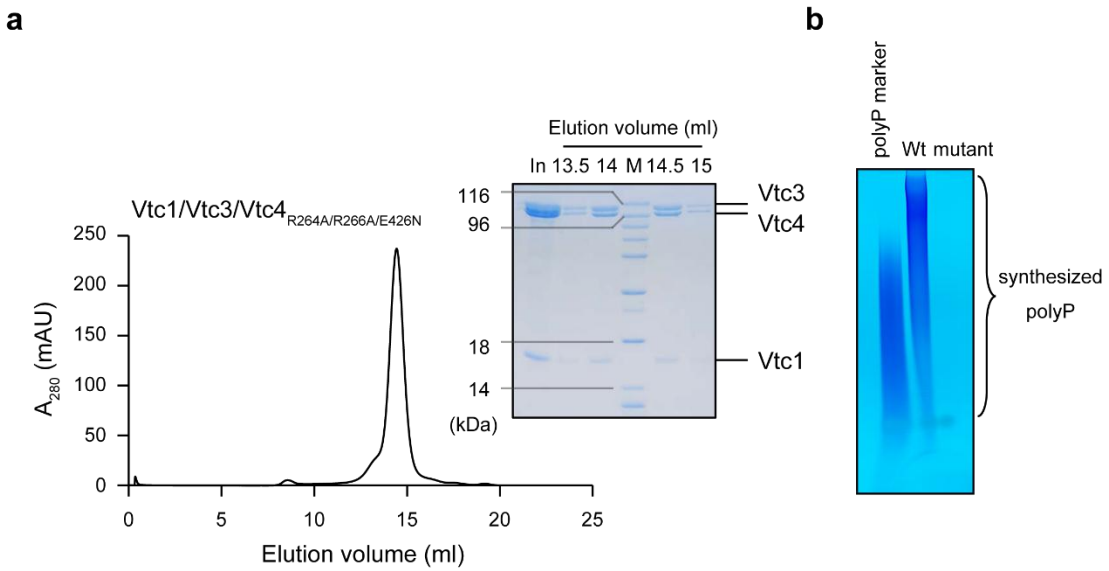


Supplementary Figure 1. (**a**) Schematic working model and (**b**) domain architectures of Vtc subunits. TM, trans-membrane domain; SPX, SYG1/Pho81/XPR1 domain; TTM, triphosphate tunnel metalloenzyme domain. A yellow star indicates the catalytic polymerase domain for polyP synthesis.



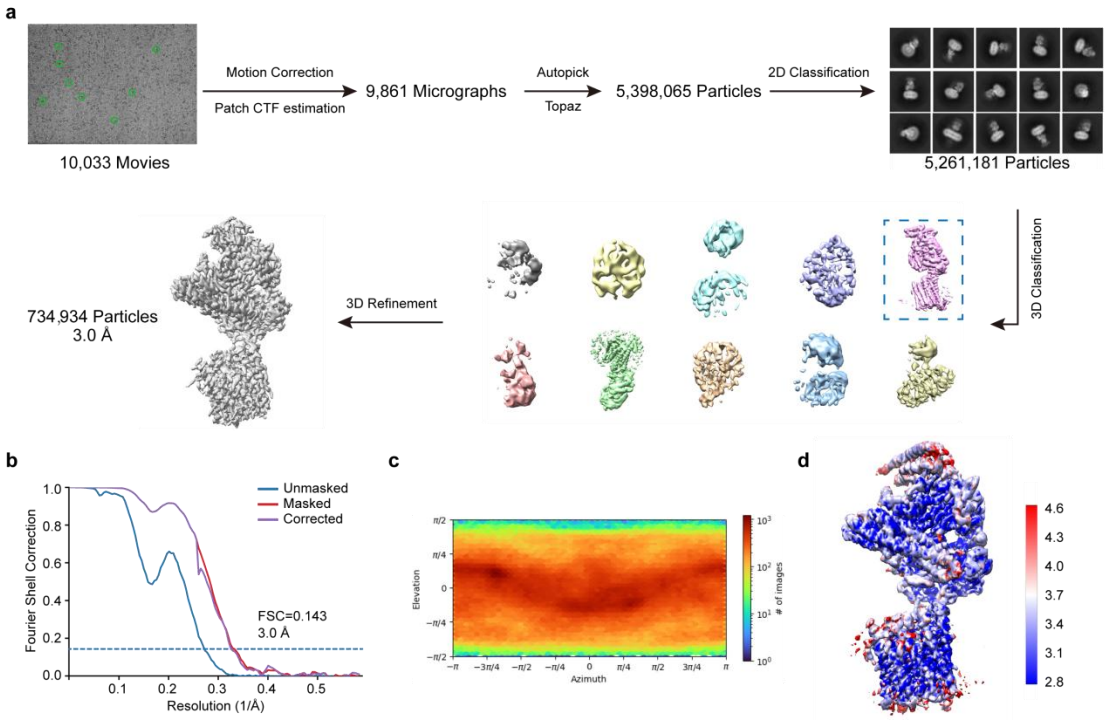
Supplementary Figure 2. Purification of the VTC complex and polyP activity assay. (**a**) Representative size-exclusion chromatography (SEC) purification of the Vtc1/Vtc3/Vtc4 complex in the absence of $InsP_6$. In, input sample before SEC step. Source data are provided as a Source Data file. The experiments were repeated more than five times independently with similar results. (**b**) TBE-PAGE visualization of polyP synthesized by the Vtc1/Vtc3/Vtc4 complex. The reactions

were performed with required times before stopped. 5 μM protein complex and 10 mM $InsP_6$ were used for each reaction. A commercial polyP with an average chain length of 60 residues (polyP60) was used as the marker. Source data are provided as a Source Data file. The experiments were repeated three times independently with similar results. (**c**) TBE-PAGE visualization of polyP synthesized by Vtc1/Vtc3/Vtc4 complex. The reactions were performed at required $InsP_6$ concentrations. 5 μM protein complex were used and reactions were performed for 4 hours. Source data are provided as a Source Data file. The experiments were repeated three times independently with similar results. (**d**) Quantification of the produced polyP from (**c)** using DAPI-based measurement[1]. PolyP60 was used for assay calibration. Data from three measurements were averaged, and the error indicates SD. Source data are provided as a Source Data file. (**e**) SEC analysis of the reaction mixture. 5 μM protein complex and 10 mM $InsP_6$ were used and the reaction of polyP synthesis was performed for 2 hours. Then the reaction mixture was subjected onto SEC. Fractions of eluent were visualized on SDS-PAGE gel. In, input reaction mixture before SEC step. CPK, creatine phosphokinase used in the reaction. Source data are provided as a Source Data file. The experiments were repeated two times independently with similar results. (**f**) DAPI detecting the retained polyP in the collected SEC fractions of the reaction mixture. Fractions of VTC at 10.5-15 ml elution volume of (**e**) were collected and concentrated for the DAPI detection. 0.2 μM concentrated protein fractions and 10 μM DAPI were used for the fluorescent detection. As controls, 0.2 μM purified VTC complex and 10 μM DAPI were used, respectively. The experiments were repeated two times independently with similar results.

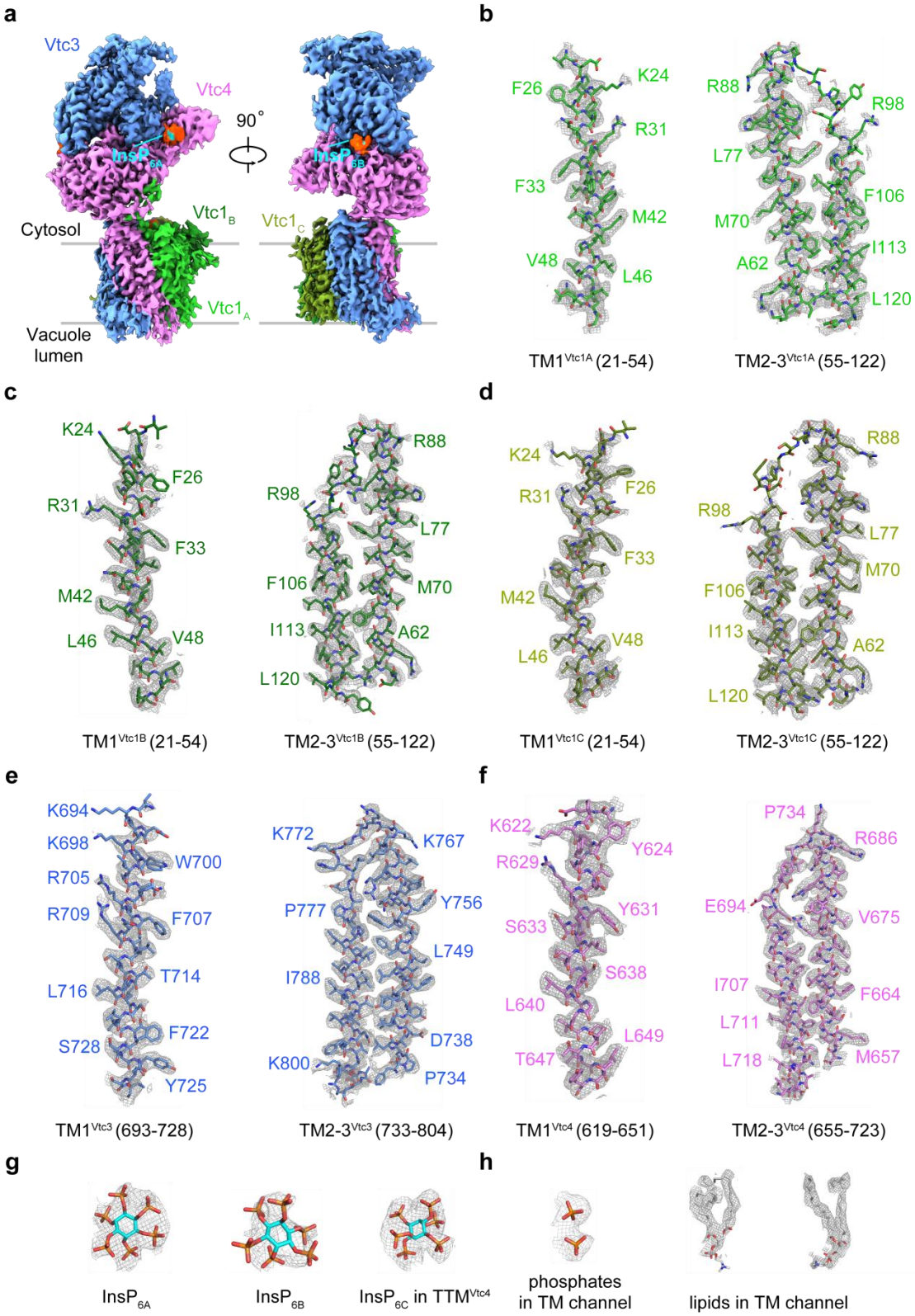


Supplementary Figure 3. Purification of the VTC complex for cryo-EM analysis. (**a**) Representative SEC purification of the Vtc1/Vtc3/Vtc4 complex carrying three point mutations on Vtc4 (Vtc1/Vtc3/$Vtc4_{R264A/R266A/E426N}$) in the presence of 1 mM $InsP_6$. In, input sample before SEC step. Fractions of Vtc1/Vtc3/$Vtc4_{R264A/R266A/E426N}$ at 14 and 14.5 ml elution volume were pooled for cryo-EM analysis. Source data are provided as a Source Data file. The experiments were repeated more than five times independently with similar results. (**b**) TBE-PAGE visualization of polyP synthesized by Vtc1/Vtc3/Vtc4 (Wt) and Vtc1/Vtc3/$Vtc4_{R264A/R266A/E426N}$

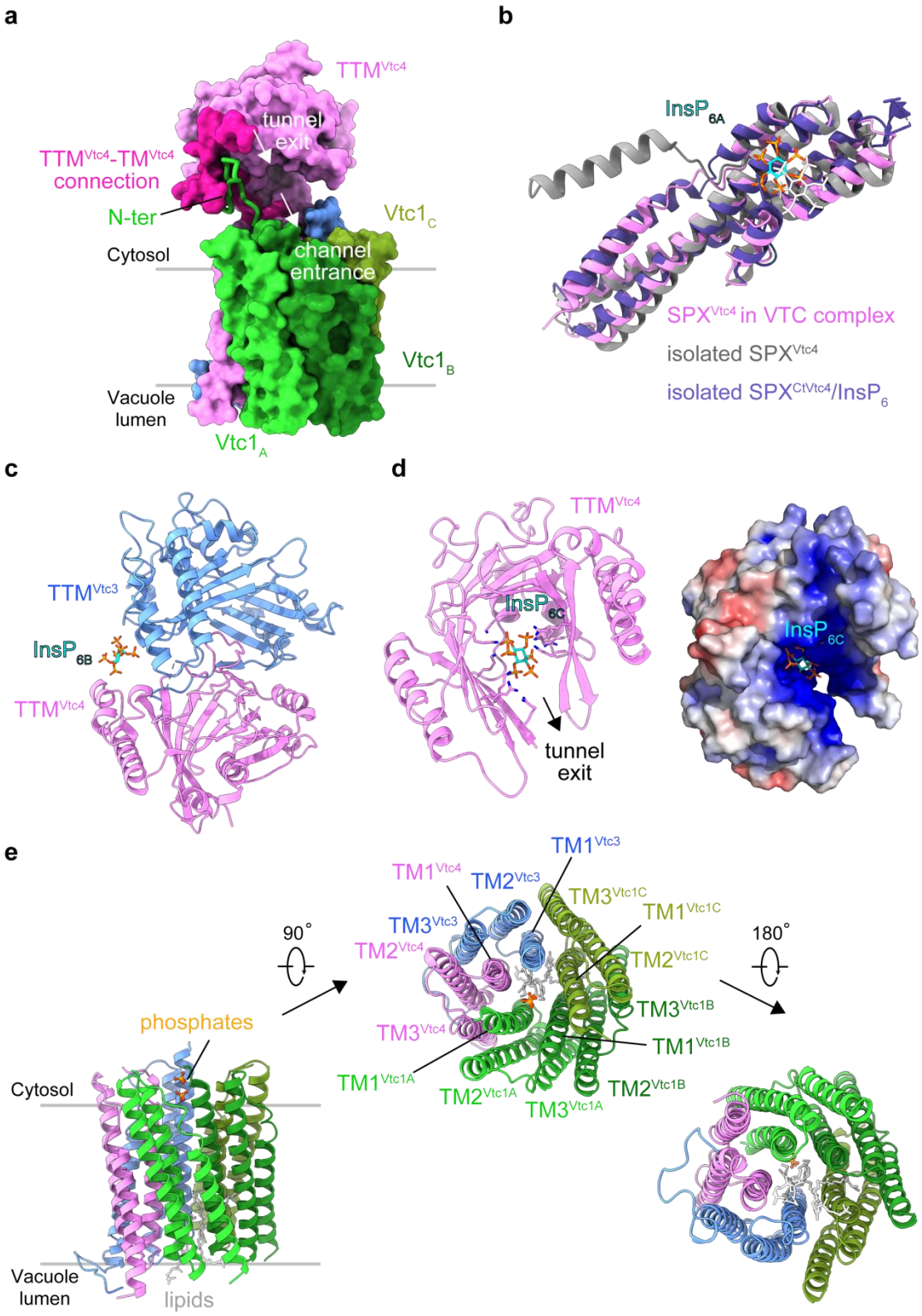
(mutant) complex. 5 μM protein complex and 10 mM $InsP_6$ were used, and reactions were performed for 4 hours. Source data are provided as a Source Data file. The experiments were repeated three times independently with similar results. Blocking VTC activity via three amino-acid substitutions of known catalytic residues in TTM^{VTC4} (R264A/R266A/E426N)[2], allowed us to collect high-quality cryo-EM micrographs for single particle analysis.



Supplementary Figure 4. Cryo-EM structural analysis of the VTC complex. (**a**) Flow chart for EM data processing. 3D refinement generated a 3.0 Å map of $Vtc1/Vtc3/Vtc4_{R264A/R266A/E426N}$ in complex with $InsP_6$. Typical particles of the complex are marked by green circles in the representative electron micrograph. Details can be found in Methods. (**b**) Gold-standard Fourier shell correlation (FSC) curve for the VTC map refinement. Average resolution of this map is estimated to be 3.0 Å. (**c**) Angular distribution of the particles used for reconstruction. (**d**) Sharpened map of the complex, colored according to local resolution.

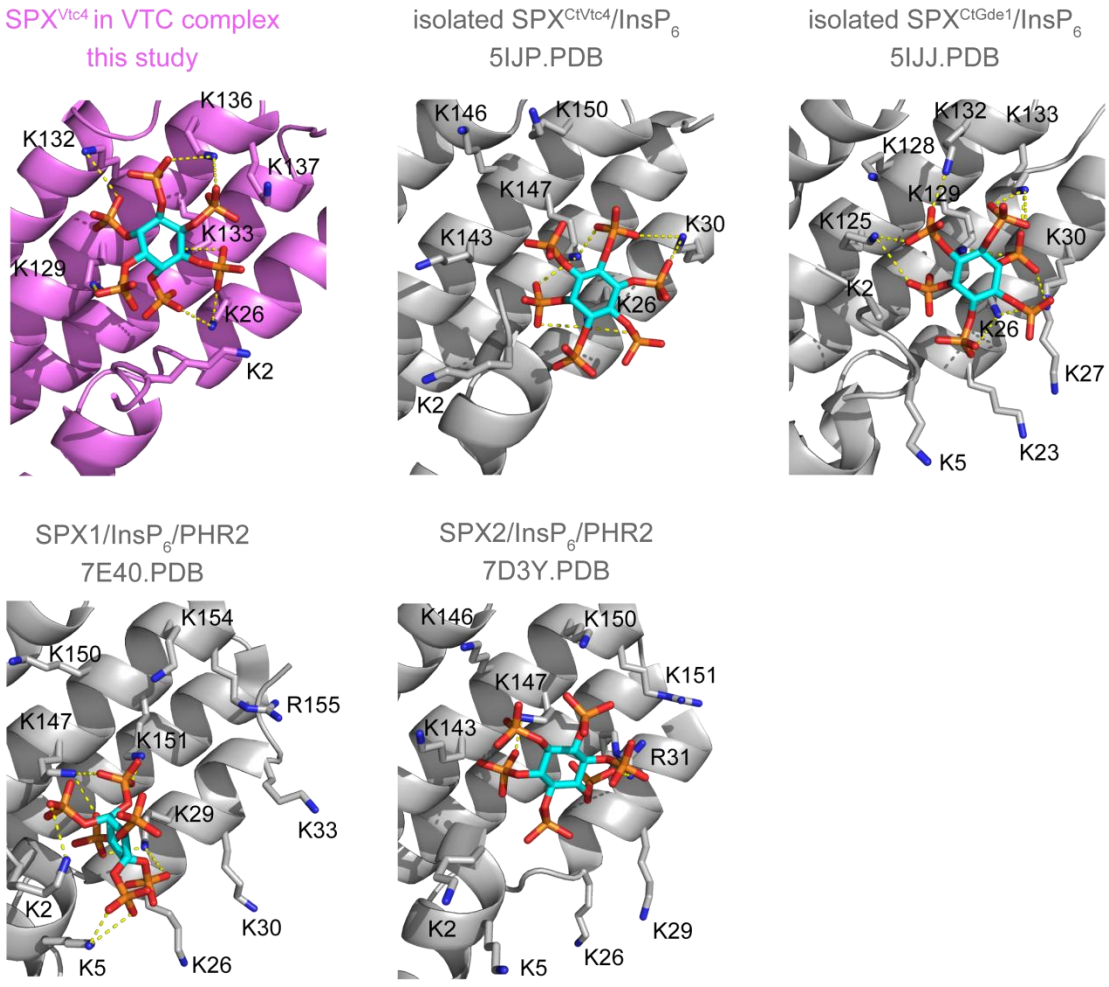


Supplementary Figure 5. Representative EM densities. (**a**) The EM density map of the Vtc1/Vtc3/Vtc4$_{R264A/R266A/E426N}$ in complex with $InsP_6$. (**b-f**) EM density maps for the indicated trans-membrane helices in the TM region of the complex. (**g**) and (**h**), EM density maps for the indicated molecules observed in the complex. All the densities were contoured at 5.5 σ and visualized using PyMol 2.4.1.

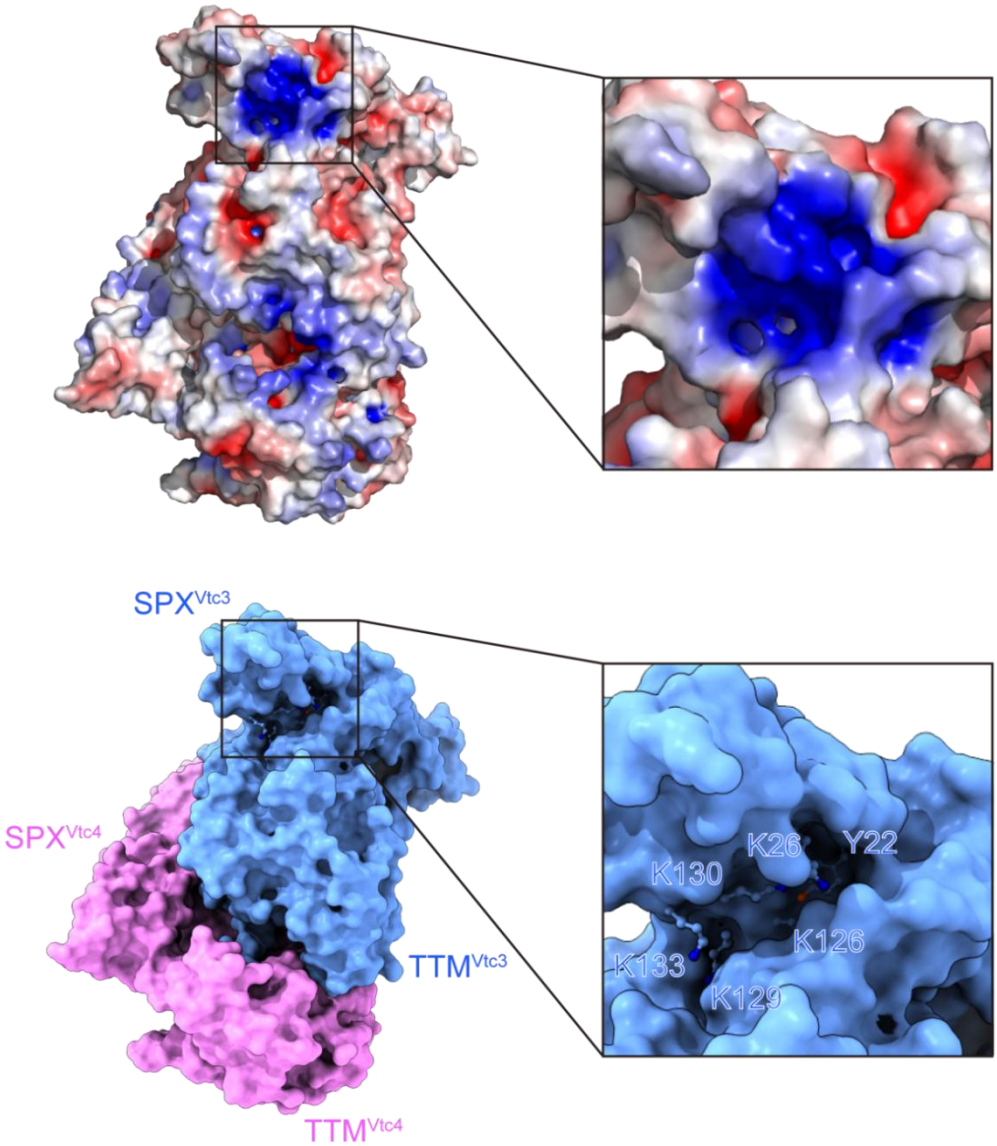


Supplementary Figure 6. Structural segments of the $InsP_6$-activated VTC complex. (**a**) Orientation between the catalytic TTM^{Vtc4} domain and the assembled trans-membrane channel. The connection between TTM^{Vtc4} and TM^{Vtc4} (residues 472-618) is represented in magenta surface. The N-terminus of $Vtc1_A$ protomer (residues 1-21) is shown in cartoon representation. (**b**) Structure comparison between $InsP_6$-bound SPX^{Vtc4} in our entire yeast VTC complex and

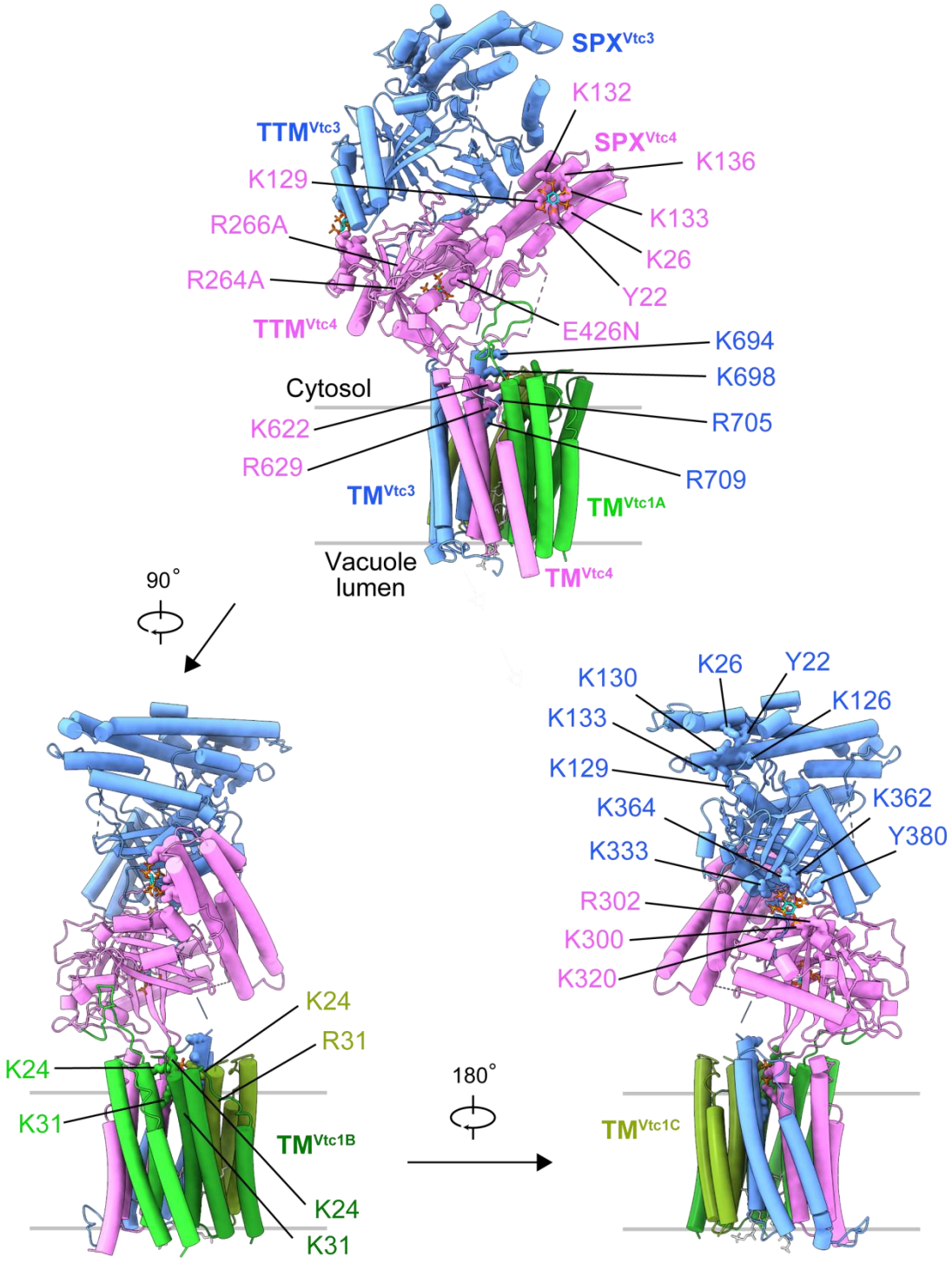
previously reported isolated SPX fragments. The structure of isolated yeast SPX^{Vtc4} fragment (5IIT.PDB)[3] and isolated *Chaetomium thermophilum* SPX^{CtVtc4} fragment in complex with $InsP_6$ (5IJP.PDB)[3] are colored in gray and purple cartoon representations, respectively. $InsP_6$ of 5IJP.PDB is shown in white stick representation. (**c**) The second $InsP_6$ molecule ($InsP_{6B}$) binds simultaneously to TTM^{Vtc3} and TTM^{Vtc4}. (**d**) The third $InsP_6$ molecule ($InsP_{6C}$) appears in the positively charged tunnel of TTM^{Vtc4}. Positive residues in inner wall of the tunnel are shown as stick in the cartoon representation of TTM^{Vtc4}. Electrostatic surface is represented in the same perspective, colored in terms of electrostatic potential and displayed in a scale from red (−5 kT/e) to blue (+5 kT/e). (**e**) Molecules of two phosphates and two lipids occupy in the cytoplasmic side and luminal side of trans-membrane channel, respectively. Phosphates and lipids are shown in stick representations.



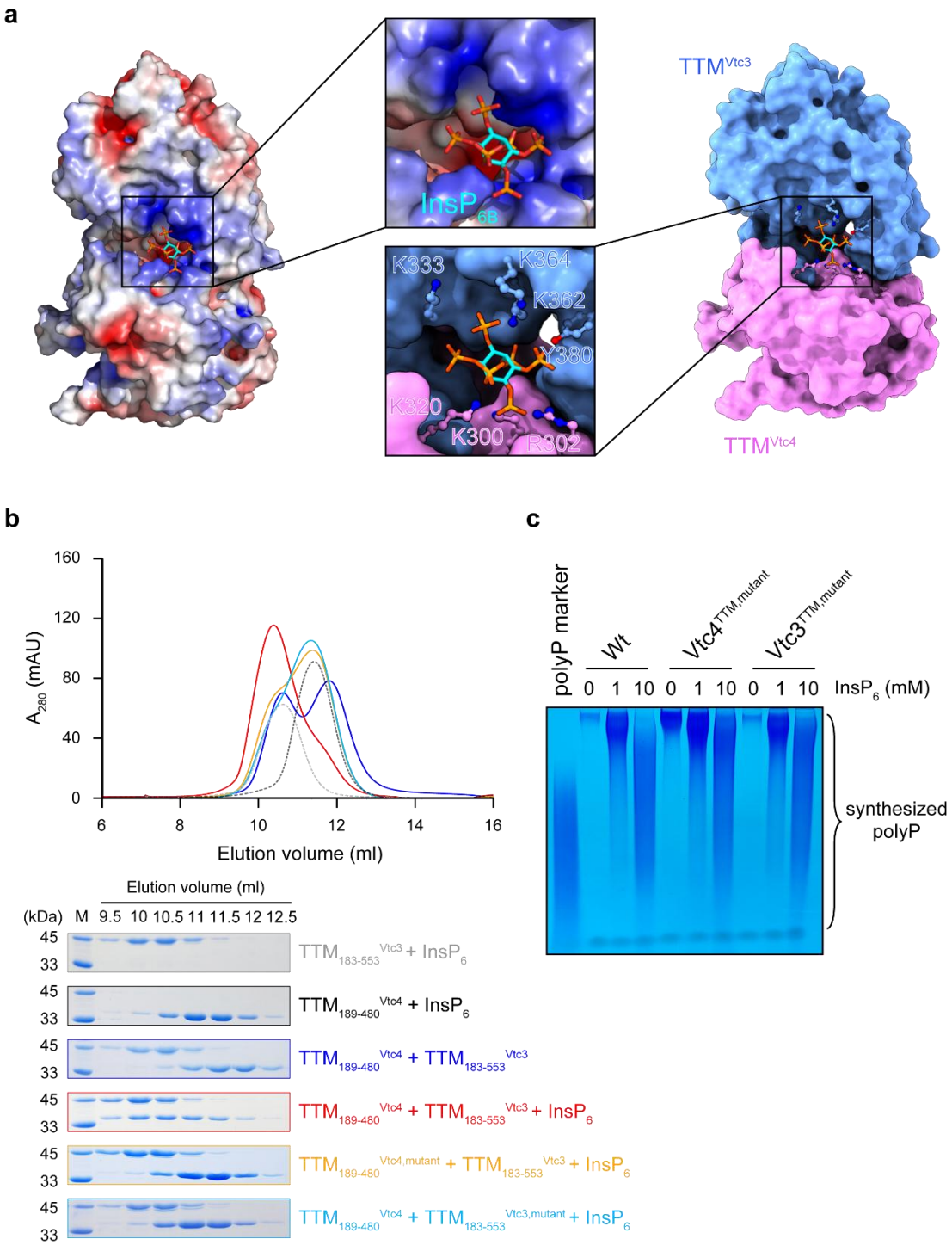
Supplementary Figure 7. Coordination of $InsP_6$ bound to different SPX domain-containing proteins. All SPX domains (depicted as cartoon diagrams) are shown in the same orientations. Other proteins in the complex structures have been omitted for clarity. Positively charged residues constituting the basic surface patch in SPX proteins are shown in stick representation. Yellow dashed lines indicate the coordination of $InsP_6$.



Supplementary Figure 8. Electrostatic surface (upper panel) and molecular surface (lower panel) representation of SPX and TTM domains in the $InsP_6$-activated VTC complex. They are displayed in the same perspective. Electrostatic potential is calculated with the APBS plugin in PyMOL, colored and displayed in a scale from red (−5 kT/e) to blue (+5 kT/e). SPX^{Vtc3} PBC (Y22, K26, K130) and KSC (K126, K129, K133) residues are shown in stick representation.

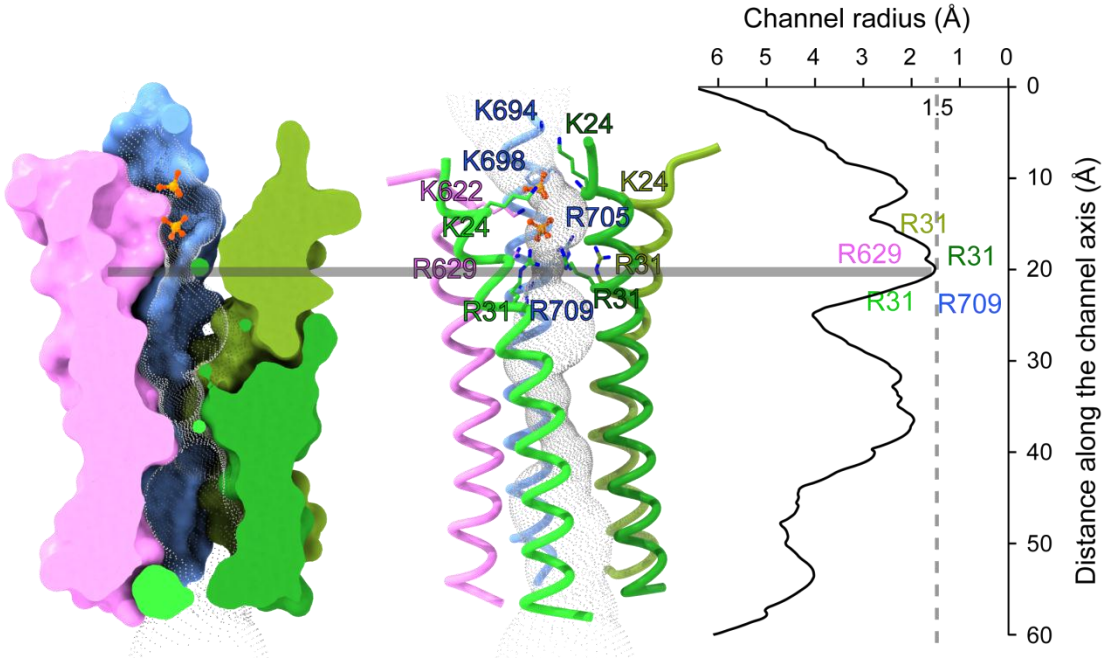


Supplementary Figure 9. Sites of point mutations used in this study. The VTC complex structure is presented in same scheme as Fig. 1 in the main text. Mutations of subunit Vtc4 R264A/R266A/E426N were used for structure determination, and others were used for function studies.

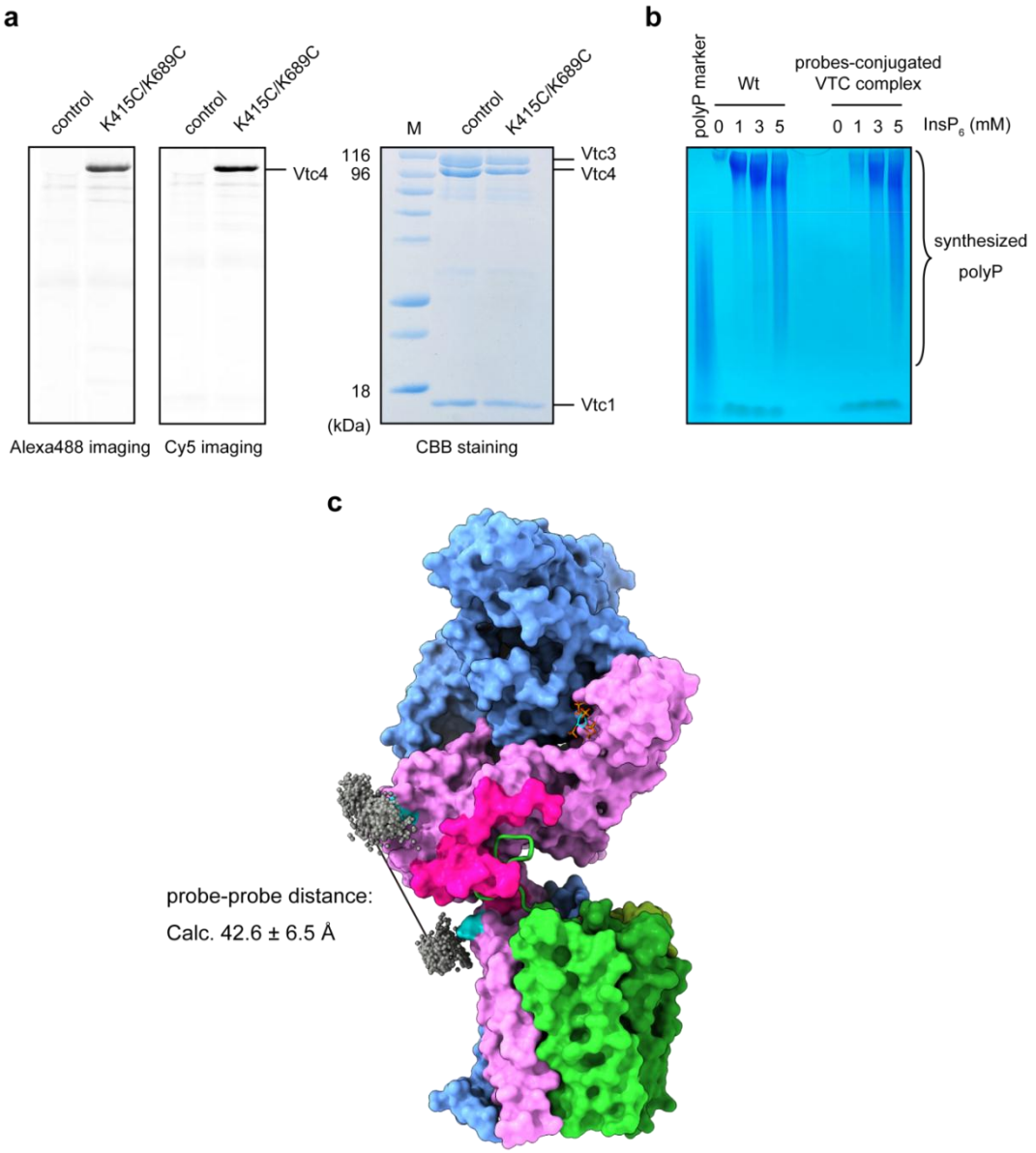


Supplementary Figure 10. An $InsP_6$ molecule is coordinated between TTM^{Vtc4} and TTM^{Vtc3} in the $InsP_6$-activated VTC complex. (**a**) $InsP_{6B}$ binds to a positively charged cleft that is formed by TTM^{Vtc4} and TTM^{Vtc3}. The Electrostatic surface (left) and molecular surface (right) representation of TTM^{Vtc3} and TTM^{Vtc4} domains are displayed in the same perspective. Electrostatic potential is calculated with the APBS plugin in PyMOL, colored and displayed in a scale from red (−5 kT/e) to blue (+5 kT/e). Residues for $InsP_6$ coordination are shown in stick representation. (**b**) $InsP_{6B}$ promotes TTM^{Vtc4}-TTM^{Vtc3} association. The isolated $TTM_{189-480}{}^{Vtc4}$ and $TTM_{183-553}{}^{Vtc3}$ contain residues 189-480 and residues 183-553, respectively. $TTM_{189-480}{}^{Vtc4,mutant}$ and $TTM_{183-553}{}^{Vtc3,mutant}$ carry K300A/R302A/K320A and K333A/K362A/K364A/Y380F point

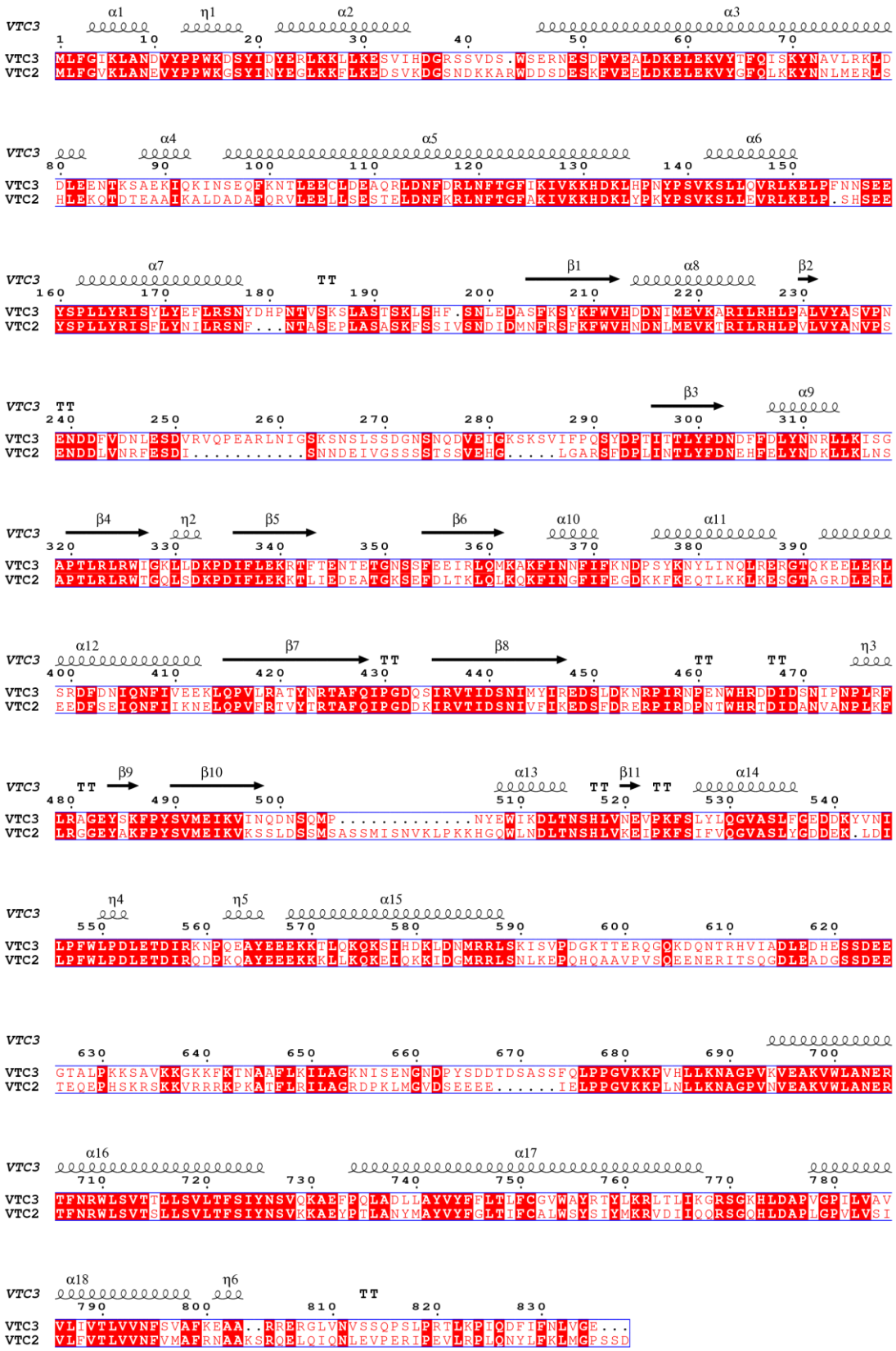
mutations, respectively. (*Top*) Gel filtration profiles of $TTM_{183\text{-}553}^{Vtc3}$, $TTM_{189\text{-}480}^{Vtc4}$, $TTM_{189\text{-}480}^{Vtc4}$ and $TTM_{183\text{-}553}^{Vtc3}$ mixture, $TTM_{189\text{-}480}^{Vtc4,mutant}$ and $TTM_{183\text{-}553}^{Vtc3}$ mixture, $TTM_{189\text{-}480}^{Vtc4}$ and $TTM_{183\text{-}553}^{Vtc3,mutant}$ mixture in the presence of 1 mM $InsP_6$, and $TTM_{189\text{-}480}^{Vtc4}$ and $TTM_{183\text{-}553}^{Vtc3}$ mixture in the absence of $InsP_6$ were colored in gray, black, red, yellow, cyan and blue, respectively. (*Bottom*) Coomassie-blue stained SDS-PAGE gels of each peak fractions. About 25 μM protein or 50 μM mixture (25 μM for each) were used for the SEC assay. Source data are provided as a Source Data file. The experiments were repeated two times independently with similar results. (**c**) Assay of VTC-catalyzed polyP synthesis. Cleft mutations in either TTM^{Vtc4} ($Vtc4^{TTM,mutant}$, $Vtc1/Vtc3/Vtc4_{K300A/R302A/K320A}$) or TTM^{Vtc3} ($Vtc3^{TTM,mutant}$, $Vtc1/Vtc3_{K333A/K362A/K364A/Y380F}/Vtc4$) had little impact on $InsP_6$-stimulated polyP synthesis by VTC complex. 5 μM protein complex were used and reactions were performed at required $InsP_6$ concentrations for 4 hours. Source data are provided as a Source Data file. The experiments were repeated two times independently with similar results.



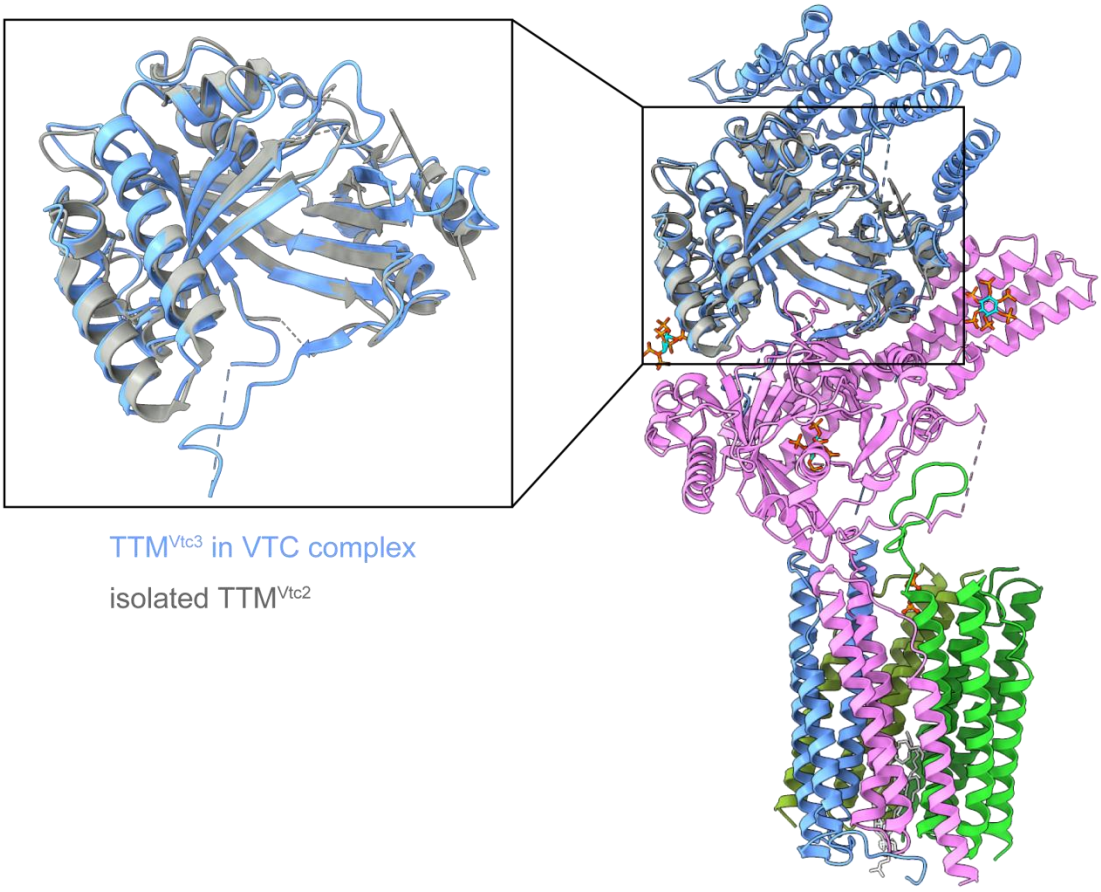
Supplementary Figure 11. Channel path analysis. Surface representation with half-cut view (left) and cartoon representation (middle) of the TM1s-assembled trans-membrane channel. Basic residues of the five TM1s in the interior wall and the occupied phosphates are shown in stick representations. Channel path (gray dots representation) and channel radius (right) was calculated using HOLE[4] for the VTC complex structure, without the inside phosphates and lipids. The dashed line indicates the narrowest position of the channel, that is surrounded by R31 of $TM1^{Vtc1}$, R709 of $TM1^{Vtc3}$, and R629 of $TM1^{Vtc4}$, with a radius of 1.5 Å. The VTC complex structure is colored in same scheme as Fig. 4b in the main text.



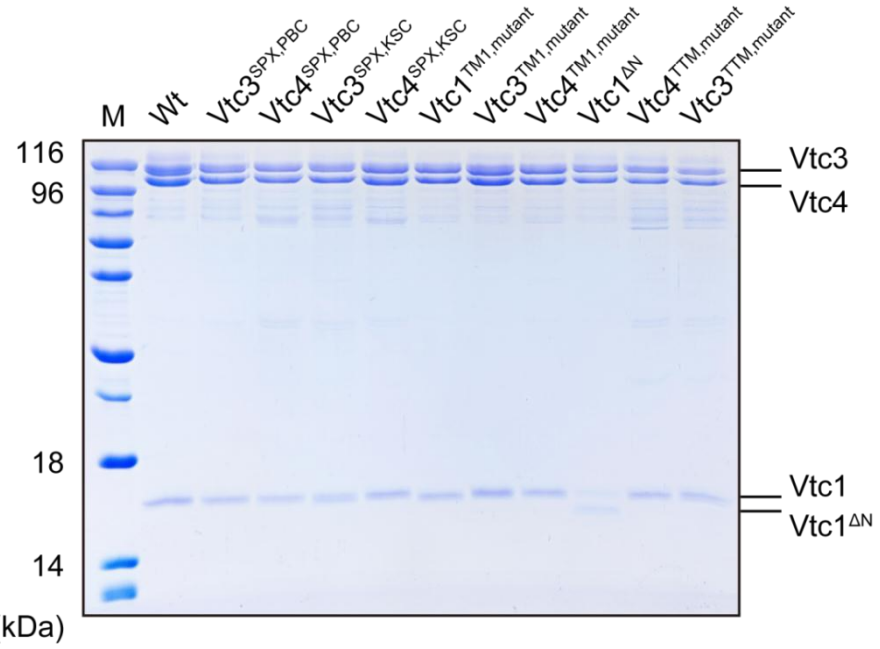
Supplementary Figure 12. Sample used for smFRET analysis. (**a**) Specifically labeling of the VTC complex. Control, all intrinsic cysteine in the Vtc1/Vtc3/Vtc4 complex were substituted; K415C/K689C, substituting all intrinsic cysteine in the VTC complex and further generating additional cysteine to K415 and K689 in the Vtc4 subunit. The images of Alexa488 and Cy5 on SDS-PAGE gel were obtained using Typhoon, and the gel was further stained using coomassie brilliant blue (CBB). Source data are provided as a Source Data file. The experiments were repeated three times independently with similar results. (**b**) Assay of VTC-catalyzed polyP synthesis. Wt, the Vtc1/Vtc3/Vtc4 wild-type complex; probes-conjugated VTC complex, the sample used for smFRET analysis. 5 μM protein complex were used and reactions were performed at required $InsP_6$ concentrations for 3 hours. Source data are provided as a Source Data file. The experiments were repeated three times independently with similar results. (**c**) Calculated average distance between conjugated probes. The accessible volume of conjugated probes on the structure were sampled using Xplor-NIH[5] (See Methods for details). The geometric centers of the two chromophores were represented with gray spheres, and the average distance between them is 42.6 ± 6.5 Å. The VTC complex structure is colored in same scheme as Fig. 5c in the main text.



Supplementary Figure 13. Sequence alignment. The sequence of yeast Vtc3 subunit is aligned with Vtc2. Structure-based alignment was performed by ESPript (3.0). The sequence identity is indicated by white letters against a red background, and the sequence of a similarity over 90% is indicated by red letters. The secondary elements of Vtc3 are labeled at the top of the alignment.



Supplementary Figure 14. Structure comparison. The structure of isolated TTM^{Vtc2} fragment[2] (3G3O.PDB) is superposed to the TTM^{Vtc3} domain in the $InsP_6$-activated Vtc1/Vtc3/Vtc4 complex structure, with an overall RMSD of 1.03 Å.



Supplementary Figure 15. Samples used for the assay of VTC-catalyzed polyP synthesis in this study. Wt, Vtc1/Vtc3/Vtc4; $Vtc3^{SPX,PBC}$, Vtc1/$Vtc3_{Y22F/K26A/K130A}$/Vtc4; $Vtc4^{SPX,PBC}$, Vtc1/Vtc3/$Vtc4_{Y22F/K26A/K133A}$; $Vtc3^{SPX,KSC}$, Vtc1/$Vtc3_{K126A/K129A/K133A}$Vtc4; $Vtc4^{SPX,KSC}$ Vtc1/Vtc3/$Vtc4_{K129A/K132A/K136A}$; $Vtc1^{TM1,mutant}$, $Vtc1_{K24A/R31A}$/Vtc3/Vtc4; $Vtc3^{TM1,mutant}$, Vtc1/$Vtc3_{K694A/K698A/R705A/R709A}$/Vtc4; $Vtc4^{TM1,mutant}$, Vtc1/Vtc3/$Vtc4_{K622A/R629A}$; $Vtc1^{\Delta N}$, $Vtc1_{\Delta 1\text{-}21}$/Vtc3 /Vtc4; $Vtc4^{TTM,mutant}$, Vtc1/Vtc3/$Vtc4_{K300A/R302A/K320A}$; $Vtc3^{TTM,mutant}$, Vtc1/$Vtc3_{K333A/K362A/K364A/Y380F}$/Vtc4. Source data are provided as a Source Data file. The experiments were repeated two times independently with similar results.

Supplementary Table 1.

Cryo-EM data collection and refinement statistics

	PDB: 7YTJ EMBD: EMD- 34090
Data collection and processing	
Microscope	Krios
Voltage (kV)	300
Camera	Gatan K3
Magnification	81,000
Pixel size at detector (Å/pixel)	0.85
Total electron exposure ($e^-/Å^2$)	55
Frames collected during exposure (no.)	32
Defocus range (μm)	-1.2~-2.2
Automation software	EPU
Micrographs collected (no.)	10,033
Micrographs used (no.)	9,861
Total extract particles (no.)	5,398,065
For each reconstruction	
Refined particles (no.)	734,934
Final particles (no.)	734,934
Point group	C1
Resolution (global, Å)	
FSC 0.5 (unmasked/masked)	4.2/3.4
FSC 0.143 (unmasked/masked)	3.7/3.0
Resolution range (local, Å)	2.8-4.6
Map sharpening B factor ($Å^2$)	132.6
Map sharpening methods	Half-maps correlation
Model composition	
Protein	1,574
Ligands	7
Model refinement	
Refinement package	PHENIX
- real or reciprocal space	Real Space
- resolution cutoff	3.0
Model-Map scores	
- CC	0.79
B factors ($Å^2$)	
Protein residues	81.12
Ligands/DNA/RNA	93.25
R.m.s. deviations from ideal values	
Bonds length (Å)	0.003

Bond angles (°)	0.637
Validation	
MolProbity score	1.74
CaBLAM outliers	2.22
Clashscore	8.42
Poor rotamers (%)	1.27
C-beta deviations	0
EMRinger score (if better than 4 Å resolution)	2.51
Ramachandran Plot	
Favored (%)	96.72
Outliers (%)	0

Supplementary Table 2.

List of yeast strains used in this study

pr, promoter

Stain name	Genotype	Background strain	Figure
Wt (BY4741)	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	*MATa*	Fig.1
*vtc1*Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vtc1::KanMX*	*BY4741*	Fig. 1
*vtc2*Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vtc2::KanMX*	*BY4741*	Fig. 1
*vtc3*Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vtc3::KanMX*	*BY4741*	Fig. 1
*vtc4*Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vtc4::KanMX*	*BY4741*	Fig. 1
*vtc1*Δ *vtc2*Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vtc1::KanMX vtc2::BleoR*	*vtc1*Δ	Fig.1
*vtc2*Δ *vtc3*Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vtc2::BleoR vtc3::KanMX*	*vtc3*Δ	
*vtc2*Δ *vtc4*Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 vtc2::BleoR vtc4::KanMX*	*vtc4*Δ	
vtc1	*vtc1*Δ*::KanMX vtc2*Δ*::BleoR vtc1* LEU2-ADHpr-N-2×Strep [pRS415-*vtc1*]	*vtc1*Δ *vtc2*Δ	Fig. 5b
$vtc1^{\Delta N}$	*vtc1*Δ*::KanMX vtc2*Δ*::BleoR vtc1* LEU2-ADHpr-N-2×Strep [pRS415-$vtc1^{\Delta N}$]	*vtc1*Δ *vtc2*Δ	Fig. 5b
$vtc1^{TM1,mutant}$	*vtc1*Δ*::KanMX vtc2*Δ*::BleoR vtc1* LEU2-ADHpr-N-2×Strep [pRS415-$vtc1^{TM1,mutant}$]	*vtc1*Δ *vtc2*Δ	Fig. 5b
vtc3	*vtc2*Δ*::BleoR vtc3*Δ*::KanMX vtc3* LEU2-ADHpr-N-Myc [pRS415-*vtc3*]	*vtc2*Δ *vtc3*Δ	Fig. 5b
$vtc3^{TM1,mutant}$	*vtc2*Δ*::BleoR vtc3*Δ*::KanMX vtc3* LEU2-ADHpr-N-Myc [pRS415-$vtc3^{TM1,mutant}$]	*vtc2*Δ *vtc3*Δ	Fig. 5b
vtc4	*vtc2*Δ*::BleoR vtc4*Δ*::KanMX vtc4* LEU2-ADHpr-N-2×Strep [pRS415-*vtc4*]	*vtc2*Δ *vtc4*Δ	Fig. 5b
$vtc4^{TM1,mutant}$	*vtc2*Δ*::BleoR vtc4*Δ*::KanMX vtc4* LEU2-ADHpr-N-2×Strep [pRS415-$vtc4^{TM1,mutant}$]	*vtc2*Δ *vtc4*Δ	Fig. 5b

Supplementary Table 3.

List of primers used in this study

Primer name	Sequence (5'-3')	Remark
Primers for yeast construction		
*vtc2*Δ (FW)	CAACATAACGACACTTTTTTGACATGGTACAGAAGATTAAGTGAG	*vtc2* deletion primer
*vtc2*Δ (Rv)	CTCAGTAGATAGAGTACATATTCTACTTTTGCTCACATGTTGGTCTC	*vtc2* deletion primer
KanMX (FW)	CCTCGACATCATCTGCCCAGATGCGAAGTTAAGTG	KanMX insert primer
KanMX (Rv)	GTTCTTTGTTGAATTTGTTGTCCACGGCTTCATCGTGTTG	KanMX insert primer
*vtc1*Δ (FW)	TCGCTATTTTCGGATAGAATGGCAAGAGCGATTAAG	upstream of *vtc1* gene
*vtc1*Δ (Rv)	CAGTTTGTGCGTAACCCACGCTTACGATATTG	downstream of *vtc1* gene
*vtc2*Δ (FW)	CACTGCGGTATAGCACTTCCAGAAAGATACTGACTG	upstream of *vtc2* gene
*vtc2*Δ (Rv)	CAAAAACCGGAGAAAATGATGTTTATGTAAATGCATTG	downstream of *vtc2* gene
*vtc3*Δ (FW)	AAGGCAGAAACGTGGAAACATACACACACGCACAC	upstream of *vtc3* gene
*vtc3*Δ (Rv)	CACCTGCTGAAAGCTGTTTAATATGATTTAATAC	downstream of *vtc3* gene
*vtc4*Δ (FW)	TGGTTTGTCTGCGTTTTGACGGAGAGCTACTGACTTGTAG	upstream of *vtc4* gene
*vtc4*Δ (Rv)	GTGTGCGCCTGGTGAAGGTGTGCATTTCAGGCAAG	downstream of *vtc4* gene
vtc1 (FW)	CCGCAGTTCGAAAAAGGATCCATGTCTTCAGCACCATTATTAC	*vtc1* complement primer
vtc1 (Rv)	GACGGTATCGATAAGCTTTTATAACTTAGTGTTAGCGTCATTG	*vtc1* complement primer
vtc2 (FW)	CAAGGATGACGACGATAAGGGATCCATGCTGTTTGGAGTGAAG	*vtc2* complement primer
vtc2 (Rv)	GACGGTATCGATAAGCTTCTAATCACTGCTTGGCCCCATTAACTTG	*vtc2* complement primer
vtc3 (FW)	CGCTCTAGAACTAGTGGATCCATGGAGCAGAAACTCATCTCTGAAG	*vtc3* complement primer
vtc3 (Rv)	GACGGTATCGATAAGCTTTTATTCCCCAACCAAATTGAAG	*vtc3* complement primer
vtc4 (FW)	CCGCAGTTCGAAAAAGGATCCATGAAGTTTGGTGAGCACTTG	*vtc4* complement primer
vtc4 (Rv)	GACGGTATCGATAAGCTTTTATTTAGCAACTAGGTTGCAGAAAAAG	*vtc4* complement primer

vtc1$_{K24A/R31A}$ (FW)	GAGTTGAGCCAGCCGTGTTCTTTGCCAATGAGGC CACCTTTTTGTCG	*vtc1*$_{K24A/R31A}$ complement primer
vtc1$_{K24A/R31A}$ (Rv)	CGACAAAAAGGTGGCCTCATTGGCAAAGAACACG GCTGGCTCAACTC	*vtc1*$_{K24A/R31A}$ complement primer
vtc1$_{\Delta 1\text{-}21}$ (FW)	TGGAGCCACCCGCAGTTCGAAAAAATGGAGCCAA AAGTGTTCTTTG	*vtc*$_{\Delta 1\text{-}21}$ complement primer
vtc1$_{\Delta 1\text{-}21}$ (Rv)	CAAAGAACACTTTTGGCTCCATTTTTTCGAACTGC GGGTGGCTCCA	*vtc*$_{\Delta 1\text{-}21}$ complement primer
vtc3$_{K694A/K698A/R705A/R709A}$ (FW)	AATGCCGGTCCTGTCGCTGTTGAGGCAGCTGTTTG GCTCGCCAAT	K694A/K698A mutation site
vtc3$_{K694A/K698A/R705A/R709A}$ (Rv)	ATTGGCGAGCCAAACAGCTGCCTCAACAGCGACA GGACCGGCATT	K694A/K698A mutation site
vtc3$_{K694A/K698A/R705A/R709A}$ (FW)	AGTTTGGCTCGCCAATGAAGCCACATTCAATGCCT GGTTAAGTGTAACCAC	R705A/R709A mutation site
vtc3$_{K694A/K698A/R705A/R709A}$ (Rv)	GTGGTTACACTTAACCAGGCATTGAATGTGGCTTC ATTGGCGAGCCAAACT	R705A/R709A mutation site
vtc4$_{K622A/R629A}$ (FW)	GTTCGTGTGGAACCAGCCGTTTACTTTGCCACTGA AGCCACCTACCTGTCTTGG	K622A/R629A mutation site
vtc4$_{K622A/R629A}$ (Rv)	CCAAGACAGGTAGGTGGCTTCAGTGGCAAAGTAA ACGGCTGGTTCCACACGAAC	K622A/R629A mutation site
Primers for prokaryotic expression		
$TTM_{189\text{-}480}^{Vtc4}$ (FW)	GAAGGAGATATACATATGGGCAAGCAGCAAAA TTTCGTGAGGCAG	$TTM_{189\text{-}480}^{vtc4}$ protein expression and purification
$TTM_{189\text{-}480}^{Vtc4}$ (Rv)	GTGATGGTGATGCTCGAGCTGGGGCAGCCAAA AGGGGATGGAG	$TTM_{189\text{-}480}^{vtc4}$ protein expression and purification
$TTM_{183\text{-}553}^{Vtc3}$ (FW)	AAGTTGATGCACATATGACCGTGTCCAAGTCCC TGGCTTCCACCAG	$TTM_{183\text{-}553}^{vtc3}$ protein expression and purification
$TTM_{183\text{-}553}^{Vtc3}$ (Rv)	CAGCCGGATCCTCGAGTTACAGGTCGGGCAGC CAAAAGGGCAG	$TTM_{183\text{-}553}^{vtc3}$ protein expression and purification
$TTM_{189\text{-}480}^{Vtc4,mutant}$ (FW)	GAAGGCCAGGTTTGCCCTGGCCGAGGCCCACG TGAATGAC	K300A/R302A mutant point

$TTM_{189-480}^{Vtc4,mutant}$ (Rv)	GTCATTCACGTGGGCCTCGGCCAGGGCAAACCTGGCCTTC	K300A/R302A mutant point
$TTM_{189-480}^{Vtc4,mutant}$ (FW)	CCAGGTGTTTGCCGCCATGAGAAAGGAGGGCAAGAAG	K320A mutation site
$TTM_{189-480}^{Vtc4,mutant}$ (Rv)	CTTCTTGCCCTCCTTTCTCATGGCGGCAAACACCTGG	K320A mutation site
$TTM_{183-553}^{Vtc3,mutant}$ (FW)	ATCGGCAAGCTGCTGGACGCCCCCGATATCTTTCTGGAG	K333A mutation site
$TTM_{183-553}^{Vtc3,mutant}$ (Rv)	CTCCAGAAAGATATCGGGGGCGTCCAGCAGCTTGCCGAT	K333A mutation site
$TTM_{183-553}^{Vtc3,mutant}$ (FW)	GGAGATCAGACTGCAGATGGCCGCTGCCTTTATCAACAATTTCATC	K362/364A mutation site
$TTM_{183-553}^{Vtc3,mutant}$ (Rv)	GATGAAATTGTTGATAAAGGCAGCGGCCATCTGCAGTCTGATCTCC	K362/364A mutation site
$TTM_{183-553}^{Vtc3,mutant}$ (FW)	GACCCTAGCTACAAGAACTTCCTGATCAACCAGCTGAG	Y380F mutation site
$TTM_{183-553}^{Vtc3,mutant}$ (Rv)	CTCAGCTGGTTGATCAGGAAGTTCTTGTAGCTAGGGTC	Y380F mutation site

Primers for eukaryotic expression

Vtc1 (FW)	ATTATCGATCCGGAGGTACCATGGCTTCCTCCGCCCCCTG	VTC1 protein expression and purification
Vtc1 (Rv)	ATGGGTAGGCGCTCTCGAGCAGCTTGGTATTGGCATC	VTC1 protein expression and purification
Vtc3 (FW)	GATGACGACGATAAGGGATCCATGCTGTTTGGCATCAAGCTG	VTC3 protein expression and purification
Vtc3 (Rv)	CTGCTAGCAAGCTTCTCGAGTTACTCGCCCACCAGGTTAAAG	VTC3 protein expression and purification
Vtc4 (FW)	ATTATCGATCCGGAGGTACCATGGCTAAGTTCGGCGAGCAC	VTC4 protein expression and purification
Vtc4 (Rv)	GGCTCCAGGCGCTCTCGAGCTTGGCCACCAGGTTAC	VTC4 protein expression and purification
$Vtc4_{R264A/R266A/E426N}$ (FW)	GAGGCCCACGCCCTGGCGTGGTACGGCGGCATG	R264A/R266A mutation site
$Vtc4_{R264A/R266A/E426N}$ (Rv)	CATGCCGCCGTACCACGCCAGGGCGTGGGCCTC	R264A/R266A mutation site

$Vtc4_{R264A/R266A/E426N}$ (FW)	TACGCCGTGCTGAACGTGAAGCTGCAGACCCAG	E426N mutation site
$Vtc4_{R264A/R266A/E426N}$ (Rv)	CTGGGTCTGCAGCTTCACGTTCAGCACGGCGTA	E426N mutation site
$Vtc3_{Y22F/K26A/K130A}$ (FW)	GATTCCTACATCGACTTCGAGAGGCTGGCTAAGCTGCTGAAGGAG	Y22F/K26A mutation site
$Vtc3_{Y22F/K26A/K130A}$ (Rv)	CTCCTTCAGCAGCTTAGCCAGCCTCTCGAAGTCGATGTAGGAATC	Y22F/K26A mutation site
$Vtc3_{Y22F/K26A/K130A}$ (FW)	TTCATCAAGATCGTGAAGGCTCACGATAAGCTGCAC	K130A mutation site
$Vtc3_{Y22F/K26A/K130A}$ (Rv)	GTGCAGCTTATCGTGAGCCTTCACGATCTTGATGAA	K130A mutation site
$Vtc4_{Y22F/K26A/K133A}$ (FW)	TACTACTACATCTCCTTCGACGACCTGGCTACAGAGCTGGAGGAC	Y22F/K26A mutation site
$Vtc4_{Y22F/K26A/K133A}$ (Rv)	GTCCTCCAGCTCTGTAGCCAGGTCGTCGAAGGAGATGTAGTAGTA	Y22F/K26A mutation site
$Vtc4_{Y22F/K26A/K133A}$ (FW)	CCAGAAGATCATCAAGGCTCACGATAAGAAGACAG	K133A mutation site
$Vtc4_{Y22F/K26A/K133A}$ (Rv)	CTGTCTTCTTATCGTGAGCCTTGATGATCTTCTGG	K133A mutation site
$Vtc3_{K126A/K129A/K133A}$ (FW)	GGCTTCATCGCTATCGTGGCTAAGCACGATGCTCTGCACCCCA	$Vtc3_{K126A/K129A/K133A}$ protein expression and purification
$Vtc3_{K126A/K129A/K133A}$ (Rv)	TGGGGTGCAGAGCATCGTGCTTAGCCACGATAGCGATGAAGCC	$Vtc3_{K126A/K129A/K133A}$ protein expression and purification
$Vtc4_{K129A/K132A/K136A}$ (FW)	GGCTTCCAGGCCATCATCGCCAAGCACGATGCCAAGACAG	$Vtc4_{K129A/K132A/K136A}$ protein expression and purification
$Vtc4_{K129A/K132A/K136A}$ (Rv)	GGCTTCCAGGCCATCATCGCCAAGCACGATGCCAAGACAG	$Vtc4_{K129A/K132A/K136A}$ protein expression and purification
$Vtc1_{K24A/R31A}$ (FW)	AGAGTGGAGCCTGCCGTGTTCTTCGCCAATGAGGCCACCTTTCTGTCCTG	$Vtc1_{K24A/R31A}$ protein expression and purification
$Vtc1_{K24A/R31A}$ (Rv)	CAGGACAGAAAGGTGGCCTCATTGGCGAAGAACACGGCAGGCTCCACTCT	$Vtc1_{K24A/R31A}$ protein

		expression and purification
$Vtc1_{\Delta1\text{-}21}$ (FW)	ATTATCGATCCGGAGGTACCATGGAGCCTAAG GTGTTCTTCGCCAAT	$Vtc1_{\Delta1\text{-}21}$ protein expression and purification
$Vtc1_{\Delta1\text{-}21}$ (Rv)	ATGGGTAGGCGCTCTCGAGCAGCTTGGTATTGG CATC	$Vtc1_{\Delta1\text{-}21}$ protein expression and purification
$Vtc3_{K694A/K698A/R705A/R709A}$ (FW)	GAACGCCGGACCTGTGGCTGTGGAGGCTGCTG TGTGGCTGGCTAATG	K694/R698A mutation site
$Vtc3_{K694A/K698A/R705A/R709A}$ (Rv)	GATGAAATTGTTGATAAAGGCAGCGGCCATCT GCAGTCTGATCTCC	K694/R698A mutation site
$Vtc3_{K694A/K698A/R705A/R709A}$ (FW)	TGTGGCTGGCTAATGAGGCTACCTTCAATGCTT GGCTGAGCGTGACAAC	R705/R709A mutation site
$Vtc3_{K694A/K698A/R705A/R709A}$ (Rv)	GTTGTCACGCTCAGCCAAGCATTGAAGGTAGCC TCATTAGCCAGCCACA	R705/R709A mutation site
$Vtc4_{K622A/R629A}$ (FW)	GTGAGAGTGGAGCCCGCCGTGTACTTTGCCACC GAGGCCACATACCTGAGCTGG	$Vtc4_{K622A/R629A}$ protein expression and purification
$Vtc4_{K622A/R629A}$ (Rv)	CCAGCTCAGGTATGTGGCCTCGGTGGCAAAGT ACACGGCGGGCTCCACTCTCAC	$Vtc4_{K622A/R629A}$ protein expression and purification
$Vtc1_{C105M}$ (FW)	GGACCTACACTGCTGATGTTTTTCCTGCTGGTG	VTC1 background Cys mutation
$VTC1_{C105M}$ (Rv)	CACCAGCAGGAAAAACATCAGCAGTGTAGGTC C	VTC1 background Cys mutation
$Vtc3_{C106M}$ (FW)	GAACACCCTGGAGGAGATGCTGGATGAGGCTC AGAG	VTC3 background Cys mutation
$Vtc3_{C106M}$ (Rv)	CTCTGAGCCTCATCCAGCATCTCCTCCAGGGTG TTC	VTC3 background Cys mutation
$Vtc3_{C751M}$ (FW)	CTTTCTGACCCTGTTCATGGGCGTGTGGGCTTA C	VTC3 background Cys mutation
$Vtc3_{C751M}$ (Rv)	GTAAGCCCACACGCCCATGAACAGGGTCAGAA AG	VTC3 background Cys mutation

$Vtc4_{C63M}$ (FW)	AAGGTGTACACATTCATGAAGGTGAAGCACAG CGAGGTG	VTC4 background Cys mutation
$Vtc4_{C63M}$ (Rv)	CACCTCGCTGTGCTTCACCTTCATGAATGTGTA CACCTT	VTC4 background Cys mutation
$Vtc4_{C418M}$ (FW)	GATGATAAGGATATCATGAGGTTCCCCTACGCC GTG	VTC4 background Cys mutation
$Vtc4_{C418M}$ (Rv)	CACGGCGTAGGGGAACCTCATGATATCCTTATC ATC	VTC4 background Cys mutation
$Vtc4_{C614M}$ (FW)	CCGGCAAGACCATCATGGTGCCTGTGAGAGTG GAG	VTC4 background Cys mutation
$Vtc4_{C614M}$ (Rv)	CTCCACTCTCACAGGCACCATGATGGTCTTGCC GG	VTC4 background Cys mutation
$Vtc4_{C716M}$ (Rv)	GGCTCCAGGCGCTCTCGAGCTTGGCCACCAGGT TCATGAAAAAGC	VTC4 background Cys mutation
$Vtc4_{K415C}$ (FW)	CTTTTAAGCAGCTGGATGATTGTGATATCTGTA GGTTCCCCTACGCC	K415C mutation site
$Vtc4_{K415C}$ (Rv)	GGCGTAGGGGAACCTACAGATATCACAATCAT CCAGCTGCTTAAAAG	K415C mutation site
$Vtc4_{R689C}$ (FW)	GGGTGGTGAACATCAGGCTGAAGTGTGCCGTG GATTACGAGGATAAGATC	R689C mutation site
$Vtc4_{R689C}$ (Rv)	GATCTTATCCTCGTAATCCACGGCACACTTCAG CCTGATGTTCACCACCC	R689C mutation site

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