

**Biochemical and structural characterization of Rab3GAP reveals insights into Rab18
nucleotide exchange activity**

Gage M.J. Fairlie^{1#}, Kha M. Nguyen^{1#}, Hannah R. Shariati¹, Sung-Eun Nam¹, Alexandria L. Shaw^{1,2}, Matthew A.H. Parson², Xinyin Wang¹, Meredith L. Jenkins², Michael Gong¹, John E. Burke^{1,2}, Calvin K. Yip^{1*}

¹Life Sciences Institute, Department of Biochemistry and Molecular Biology, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3

²Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada V8W 2Y2

[#]These authors contributed equally: Gage M.J. Fairlie, Kha M. Nguyen

***Corresponding author: Calvin K. Yip**

e-mail: calvin.yip@ubc.ca

Supplementary Information

Supplementary Figure 1. Rab3GAP GEF assays with Rab18 and Rab11a

Supplementary Figure 2. AlphaFold model of Rab3GAP2 used to generate core Rab3GAP

Supplementary Figure 3. Cryo-EM data processing workflow of core Rab3GAP dataset

Supplementary Figure 4. Rab3GAP subunit interfacial interactions

Supplementary Figure 5. HDX-MS analysis comparing Rab3GAP1 to FL Rab3GAP complex

Supplementary Figure 6. AlphaFold3 predictions of core Rab3GAP with Rab18

Supplementary Figure 7. Multiple sequence alignment of predicted interacting residues from AlphaFold model of Rab3GAP1/2 and Rab18

Supplementary Figure 8. WT Rab18 and Rab18 E164A mutant localization in HeLa cells

Supplementary Figure 9. Multiple sequence alignment of the Rab subfamily of GTPases

Supplementary Figure 10. AlphaFold3 predictions of Rab3GAP1 and Rab3a

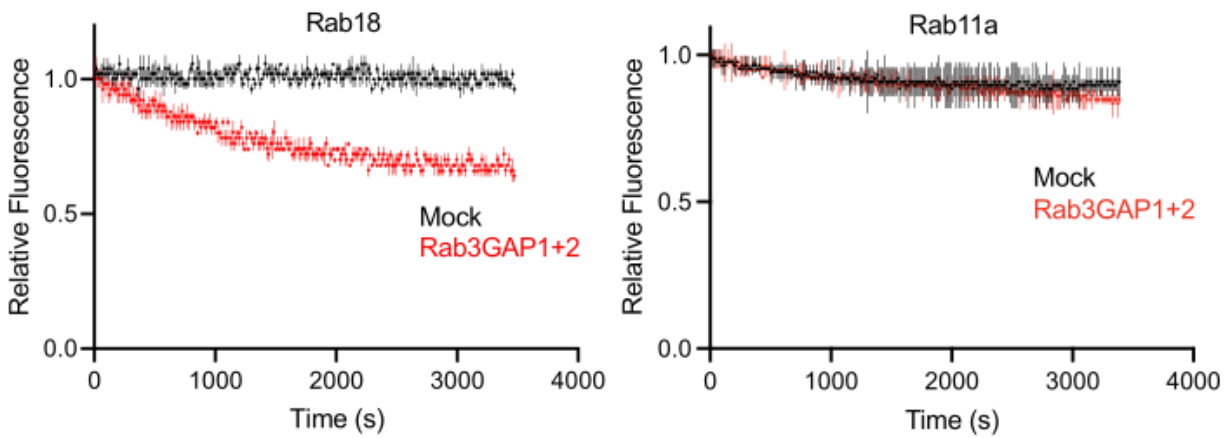
Supplementary Figure 11. Comparison of previously solved Rab-GEF structures to the AlphaFold3 predicted Rab3GAP-Rab18 complex

Supplementary Figure 12. Cluster of basic residues on the AlphaFold2-predicted structural model C-terminal domain of Rab3GAP2

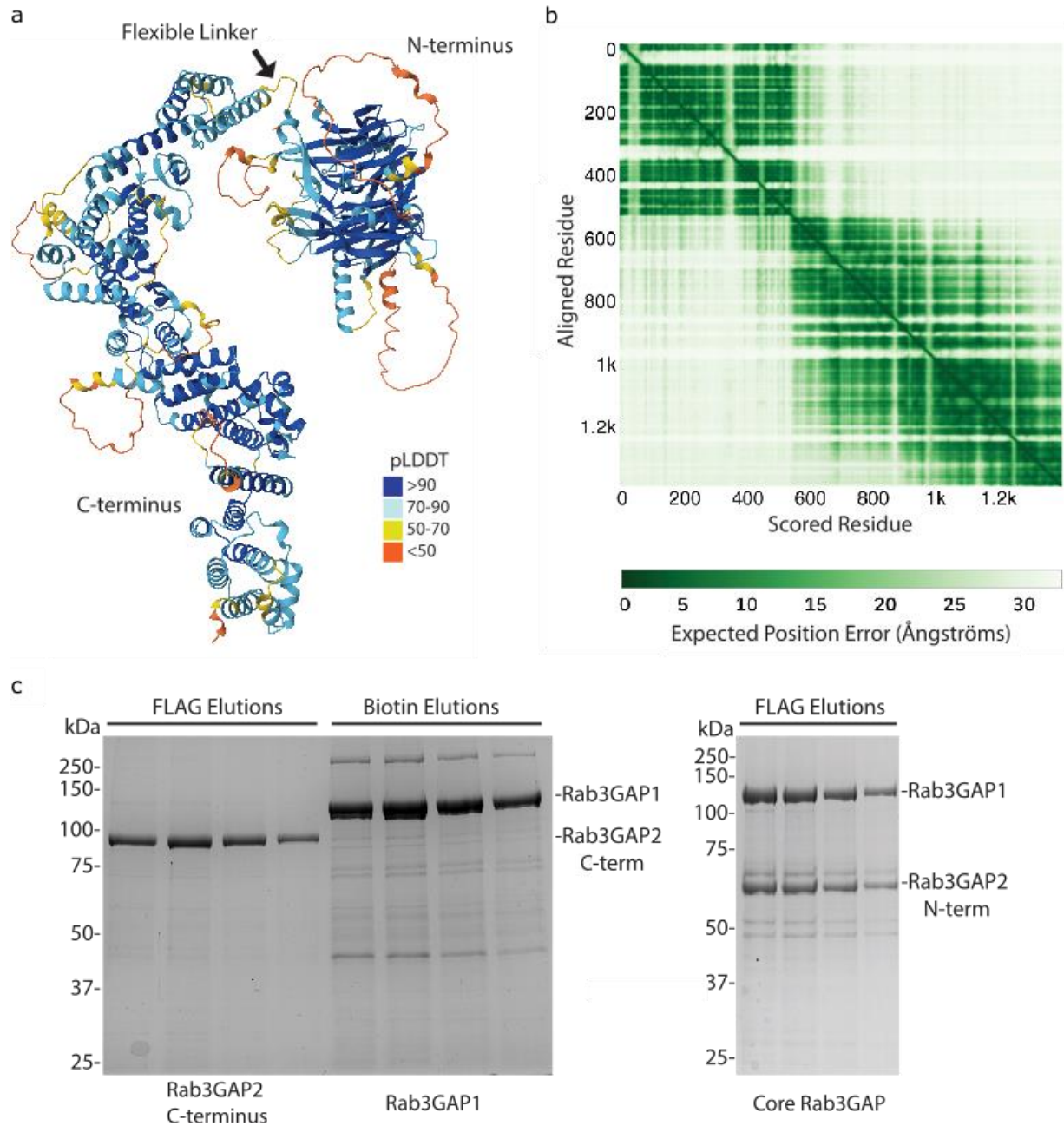
Supplementary Table 1. Description of plasmids used in this study

Supplementary Table 2. Vesicle composition used for in vitro GEF assays

Supplementary Table 3. Cryo-EM structural and refinement statistics



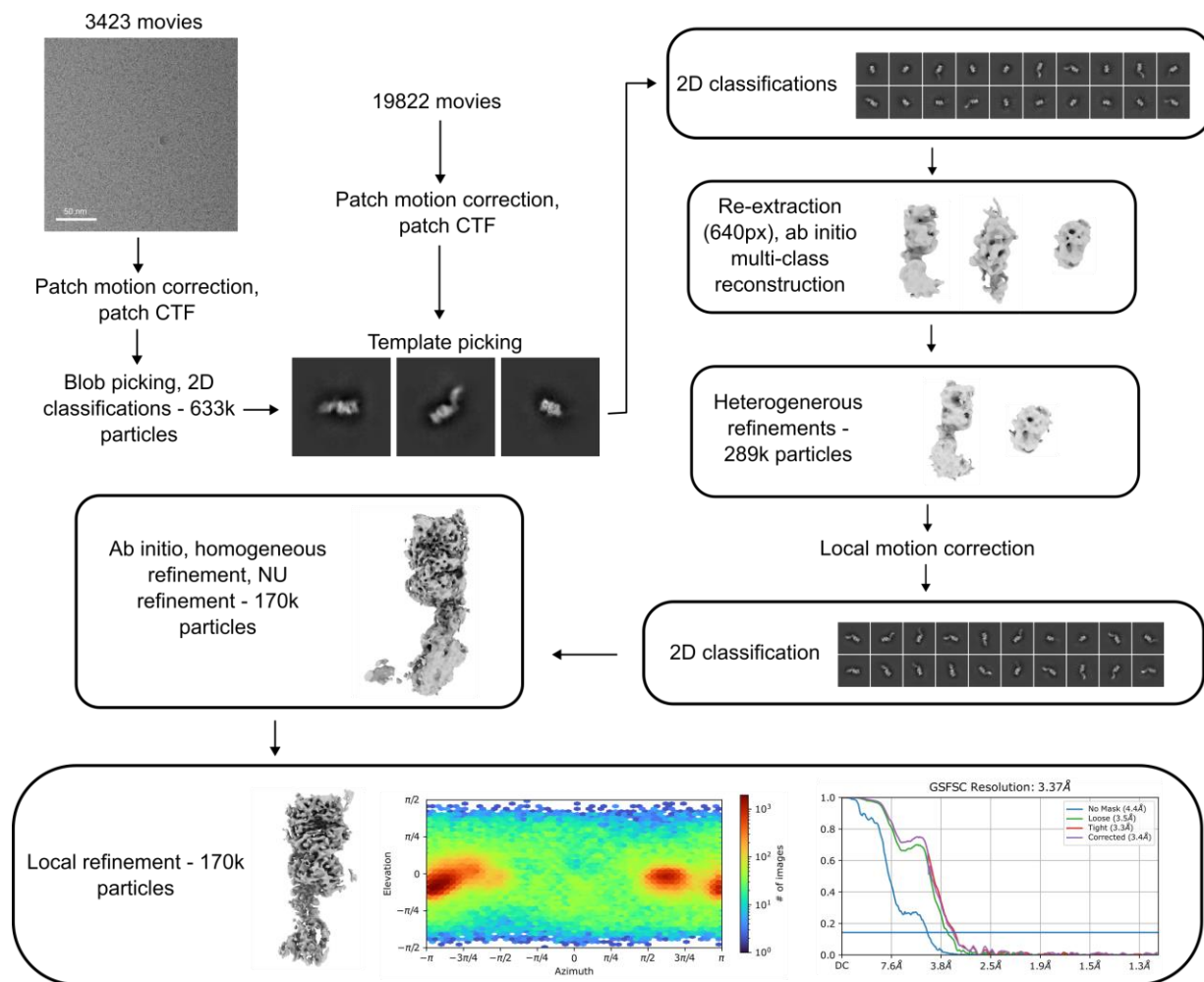
Supplementary Figure 1. GEF assays on Rab18 and Rab11a with Rab3GAP. Nucleotide exchange was detected by measuring fluorescent decrease in reactions containing 0 nM GEF (Mock) or 300 nM GEF with 4 μ M Mant-GDP loaded Rab and 100 μ M GTP γ S. Data is presented as mean with error bars showing SEM for assays performed in technical triplicate (n=3). Source data are provided as a Source Data file.



Supplementary Figure 2. AlphaFold model of Rab3GAP2 used to generate core Rab3GAP.

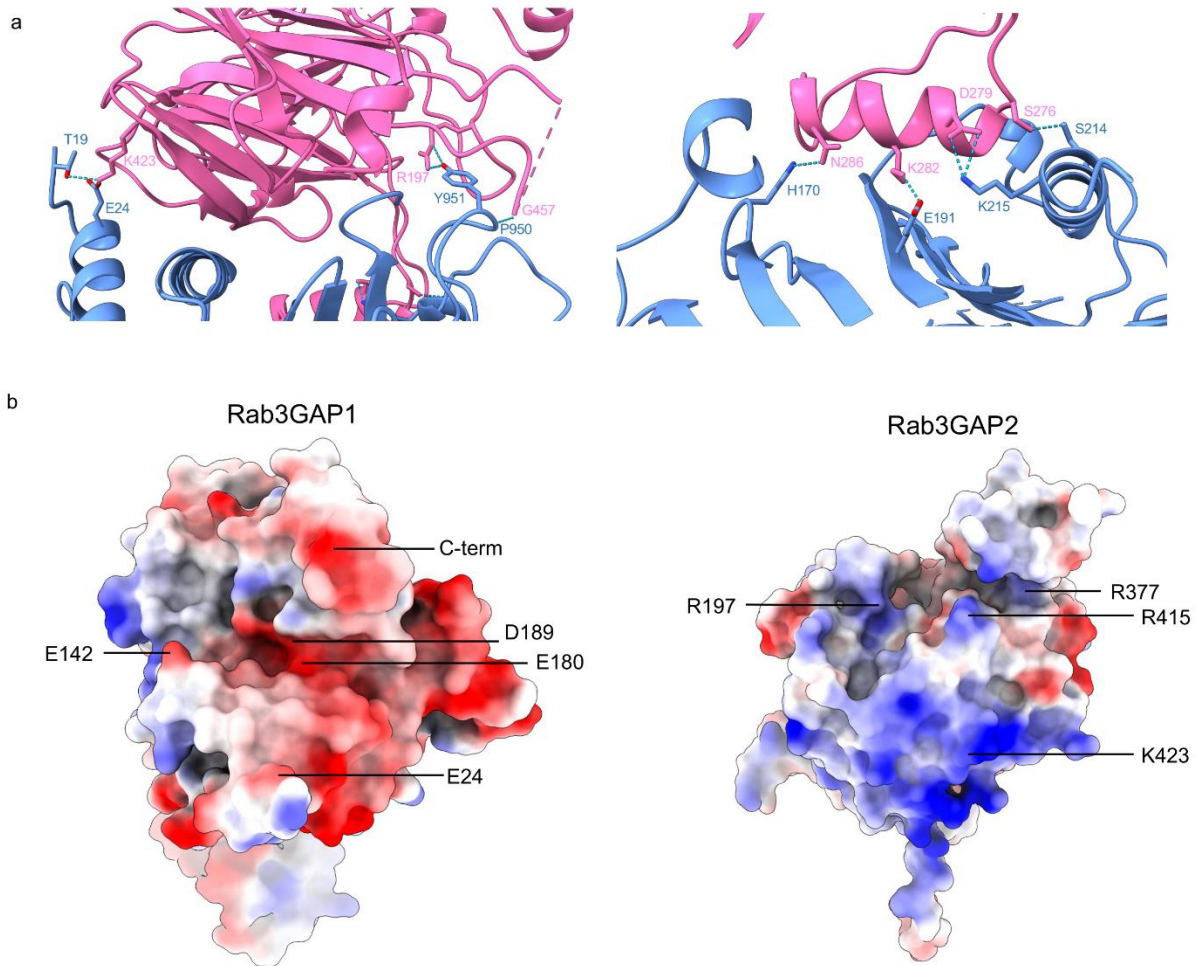
a. Predicted structure of Rab3GAP2 obtained from the AlphaFold database showing N-terminal and C-terminal domains connected through a flexible linker. Rab3GAP2 is coloured according to pLDDT score. **b.** Predicted alignment error (PAE) plot for Rab3GAP2 structural prediction from the AlphaFold database. **c.** Affinity chromatography elutions of Rab3GAP1-Rab3GAP2C and

Rab3GAP1-Rab3GAP2N stained with Coomassie Blue on an SDS-PAGE gel. Purifications and SDS-PAGE gels were performed in biological triplicate (n=3). Source data are provided as a Source Data file.

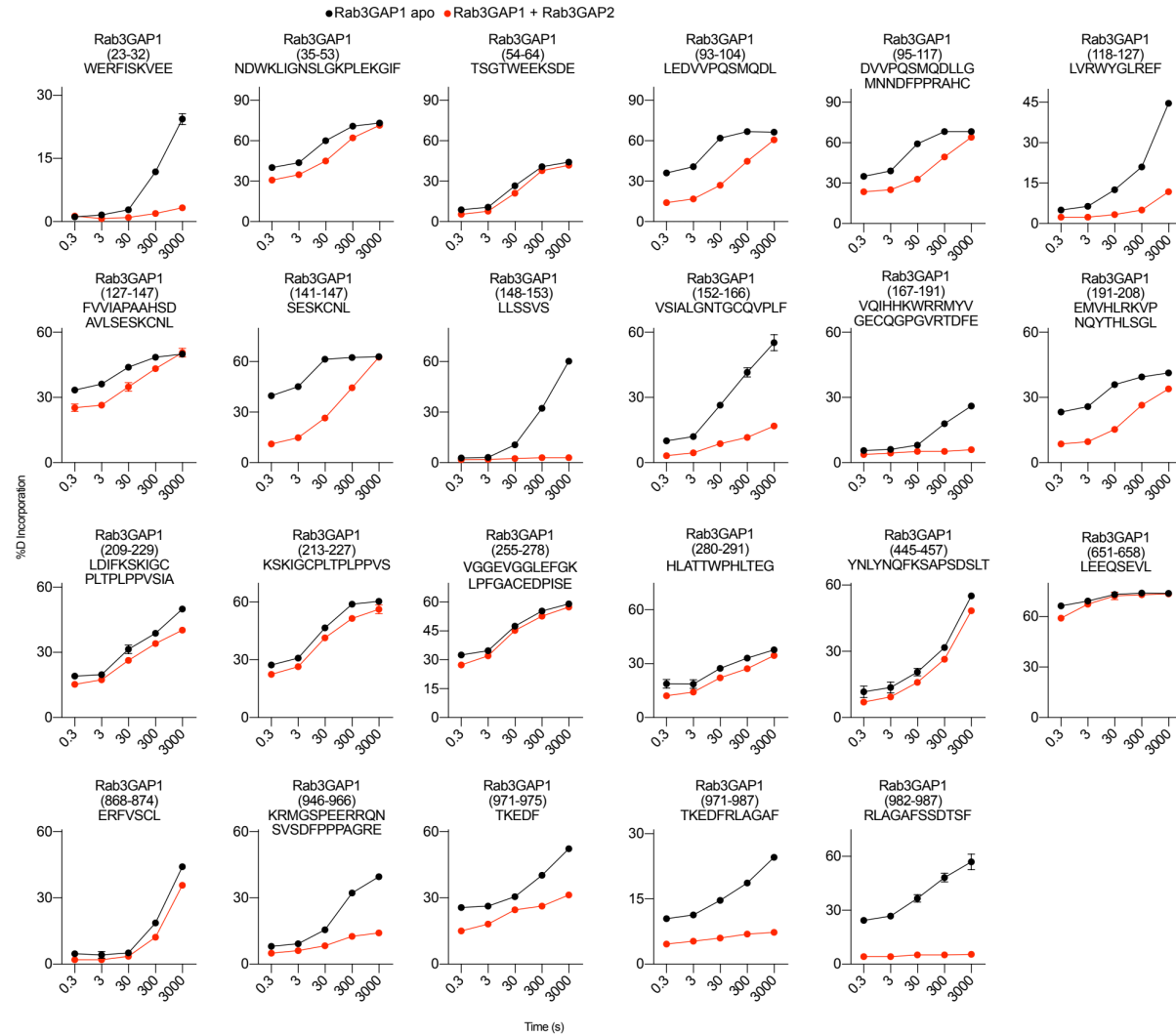


Supplementary Figure 3. Cryo-EM data processing workflow of core Rab3GAP dataset.

The CryoEM data collection and processing workflow of core Rab3GAP including 2D class averages, 3D reconstructions, local refinement and final reconstruction at different stages of data processing are displayed. Angular distribution of particles used in the final reconstruction and Gold-standard Fourier Shell Correlation (FSC) curves of the final reconstruction generated in cryoSPARC are shown.



Supplementary Figure 4. Rab3GAP subunit interfacial interactions. **a.** Hydrogen bond pairs between residues on both Rab3GAP subunits. Rab3GAP1 in blue and Rab3GAP2N in pink **b.** Negative and positive electrostatic potential of the Rab3GAP1 and Rab3GAP2 subunits. Blue color represents positive charge, white for neutral, and red for negative charge.

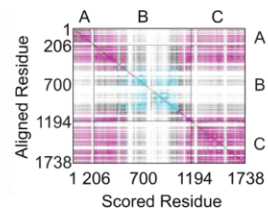
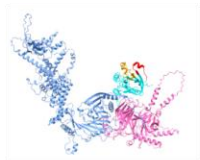


Supplementary Figure 5. HDX-MS analysis comparing Rab3GAP1 to FL Rab3GAP

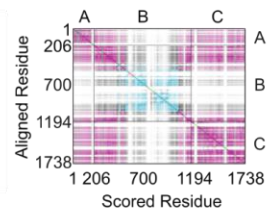
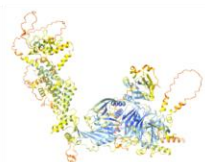
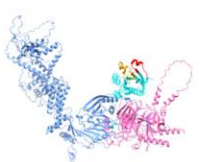
complex. HDX-MS time courses for selected peptides with significant differences (defined as $>5\%$, >0.4 Da, and $p < 0.01$ in an unpaired two-tail t test at any time point). Selected deuterium exchange time courses of Rab3GAP1 peptides representing all regions that showed significant decreases and increases in exchange upon complex formation. Data are presented as mean values with error bars representing SD from experiments performed in technical triplicate ($n=3$).

a

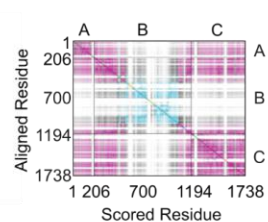
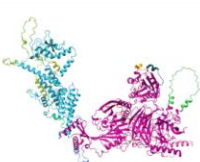
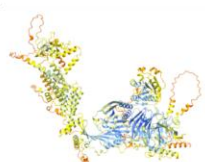
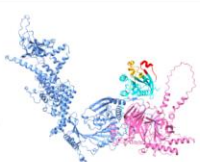
Model 0
pTM: 0.68
ipTM: 0.8
Ranking score: 0.86



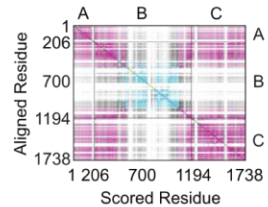
Model 1
pTM: 0.68
ipTM: 0.8
Ranking score: 0.85



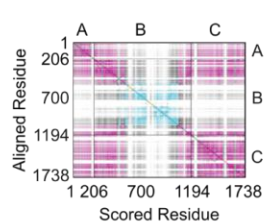
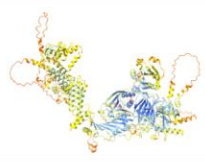
Model 2
pTM: 0.68
ipTM: 0.8
Ranking score: 0.85



Model 3
pTM: 0.68
ipTM: 0.8
Ranking score: 0.86



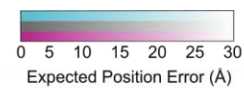
Model 4
pTM: 0.69
ipTM: 0.8
Ranking score: 0.85



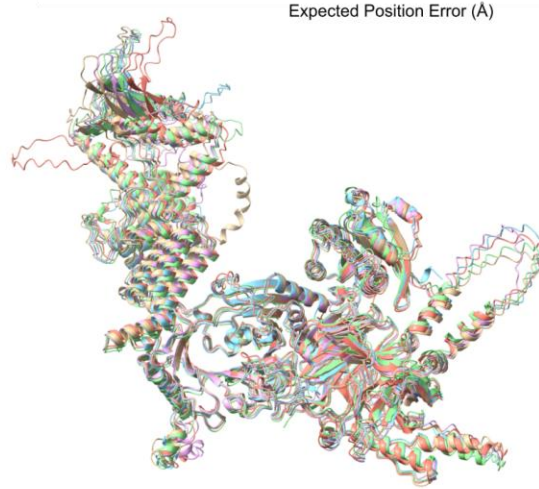
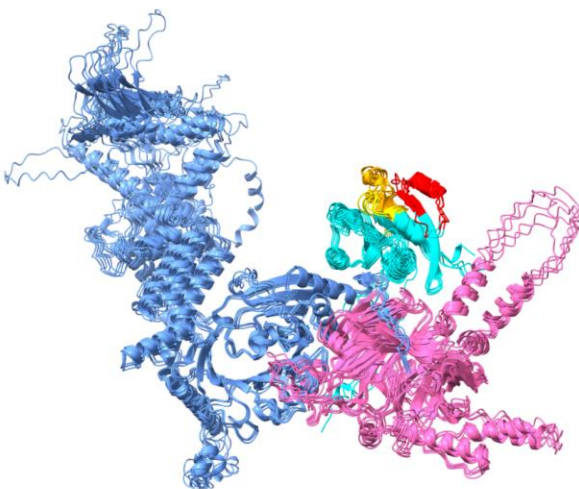
Rab3GAP1
Rab3GAP2 N
Rab18

Switch I
Switch II
P-loop

pLDDT
■ >90 ■ 70-90
■ 50-70 ■ <50

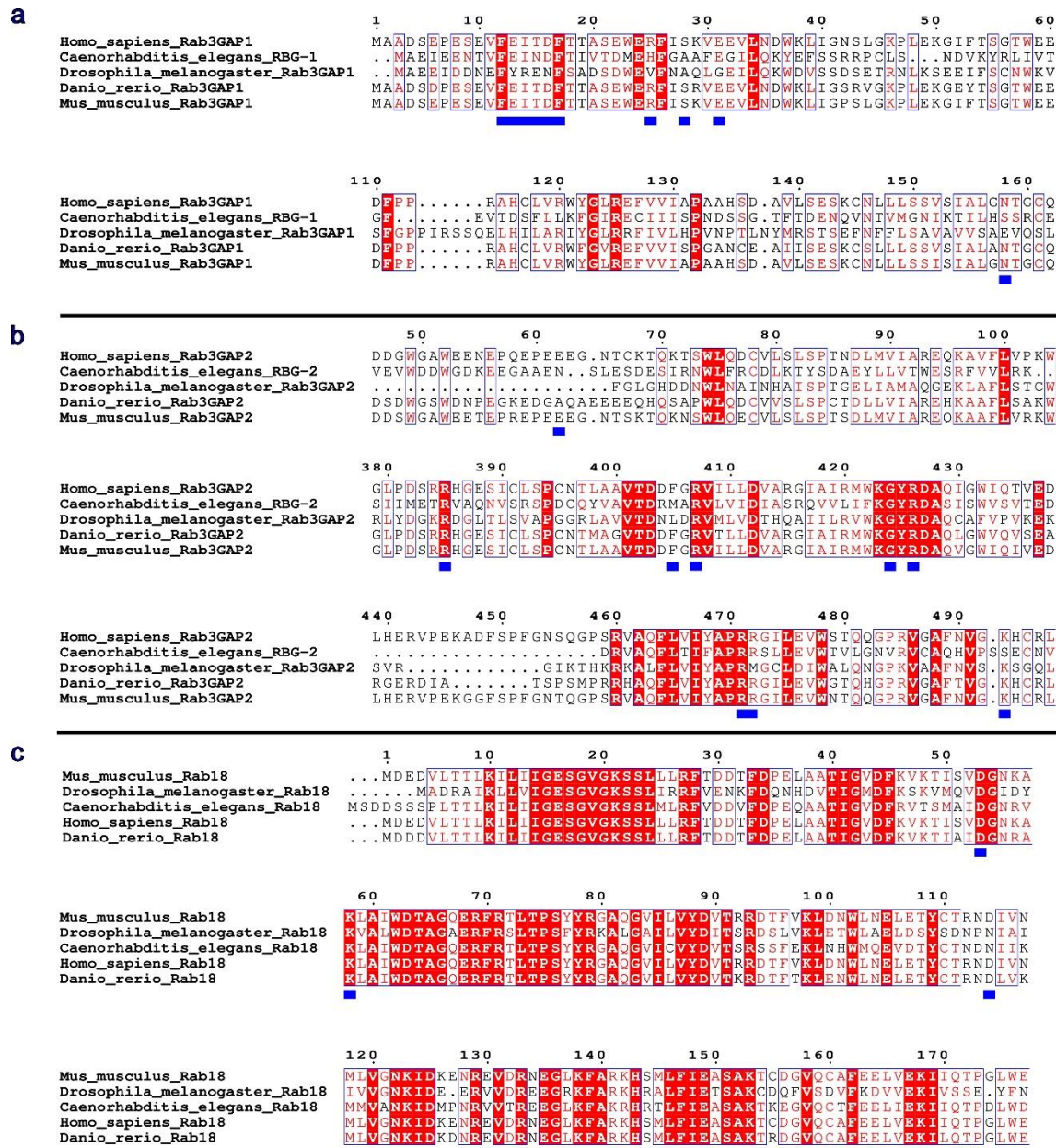


b



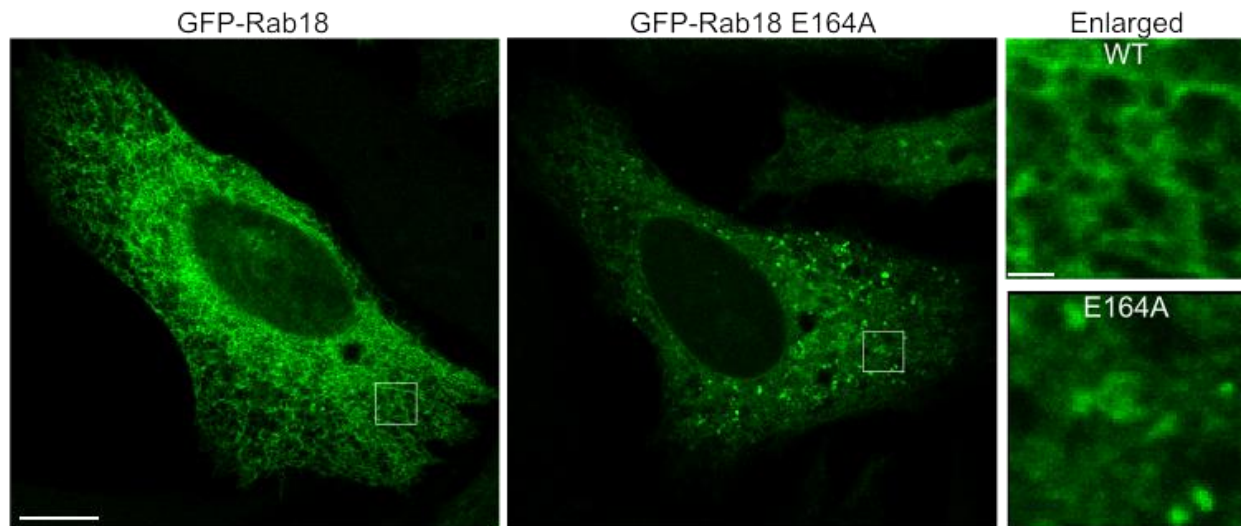
Supplementary Figure 6. AlphaFold predictions of core Rab3GAP apo and with Rab18. a.

AlphaFold3 prediction of core Rab3GAP (Rab3GAP1 in blue and Rab3GAP2N in pink) in complex with Rab18 (Rab18 in cyan with switch I, switch II and p-loop coloured in red, orange and yellow respectively). pTM, iPTM and overall ranking score are displayed for all models, and pLDDT scores are mapped by coloured onto each model. PAE plots for each prediction are displayed with chain A, chain B and chain C corresponding to Rab18, Rab3GAP1 and Rab3GAP2N respectively. ChimeraX PAE domain feature was used to colour models based on pseudo-rigid domains with PAE cutoff set to less than 5 angstroms. Rab18 and the Rab3GAP1-Rab3GAP2N interface form one PAE domain (purple) and the Rab3GAP1 tail form another PAE domain (light blue). **b.** All AlphaFold3 models of core Rab3GAP bound to Rab18 superimposed for comparison using the same colour scheme as above or coloured individually according to model number.



Supplementary Figure 7. Multiple sequence alignment of predicted interacting residues from AlphaFold3 model of Rab3GAP1/2 and Rab18. a. Multiple sequence alignment of Rab3GAP1 predicted interacting residue to Rab18. **b.** Multiple sequence alignment of Rab3GAP2 predicted interacting residue to Rab18. **c.** Multiple sequence alignment of Rab18 predicted interacting residues to Rab3GAP. Red box with white font indicates strict

conservation, red character indicates similarity within a group and a blue frame indicates similarity across groups. Predicted interacting residues are indicated by blue boxes.

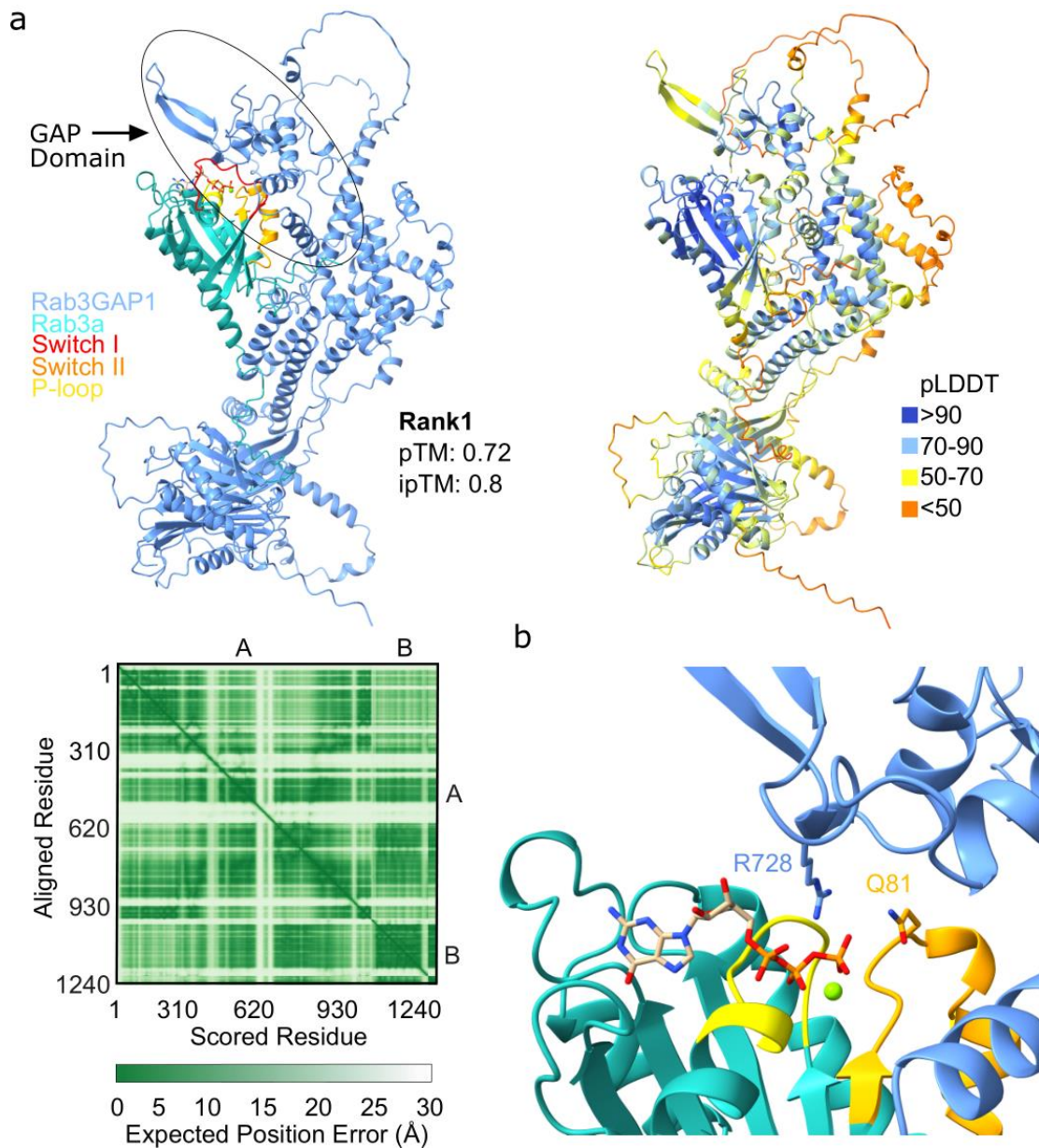


Supplementary Figure 8. Rab18 localization is disturbed by E164A mutation. HeLa cells expressing GFP-Rab18 or GFP-Rab18 E164A. Enlarged regions corresponding to the white boxes show details of Rab18 localization. Scale bars are 10 μm long in overview and 1 μm long in enlarged view. Experiments were performed in biological triplicate (n=3). Source data are provided as a Source Data file.



Supplementary Figure 9. Multiple sequence alignment of the Rab subfamily of GTPases.

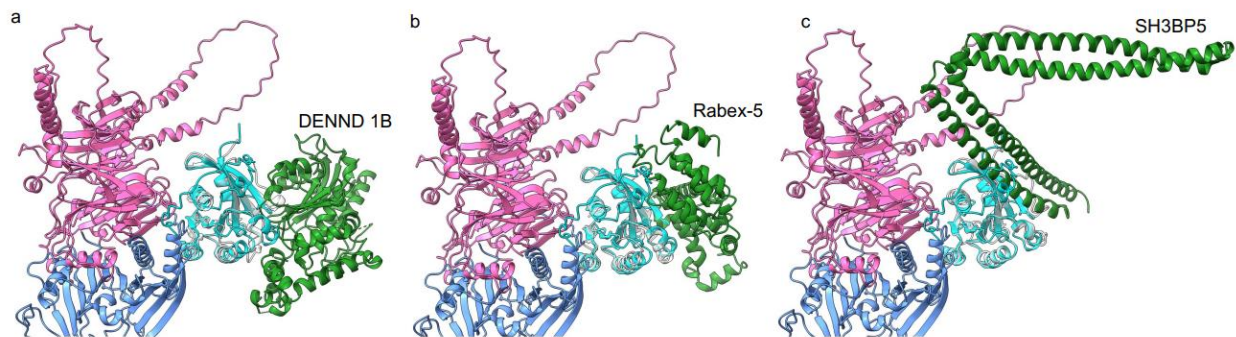
Multiple sequence alignment of Rab subfamily of small GTPases to Rab18 at the switch I, switch II and p-loop region, as well as the Rab3GAP binding interface on Rab18. Red box with white font indicates strict conservation, red character indicates similarity within a group and a blue frame indicates similarity across groups. Residues critical to mediating Rab18 activation by Rab3GAP (R133, R141 and E164) are indicated by black arrows.



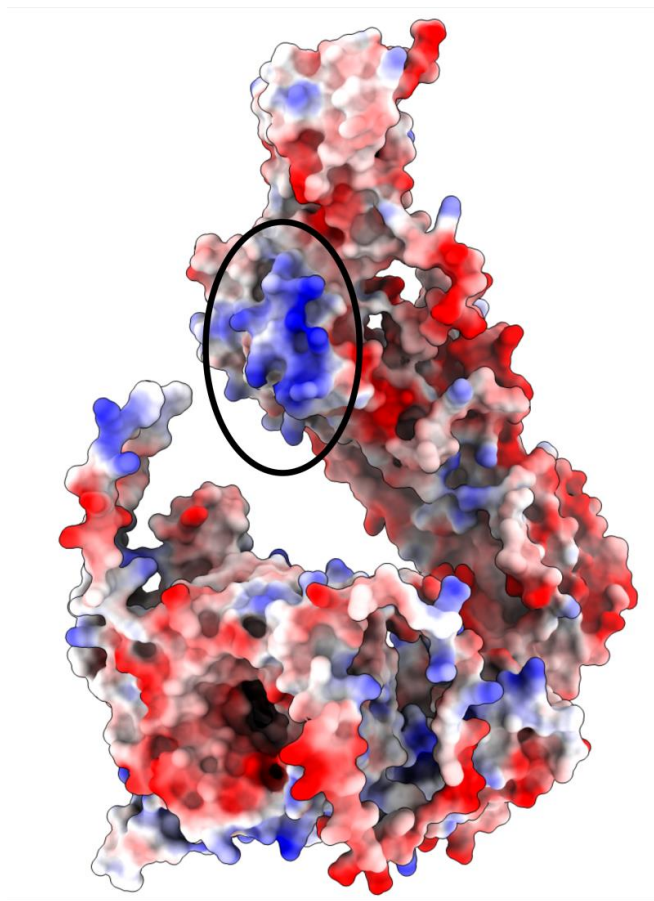
Supplementary Figure 10. Core Rab3GAP with a GAP domain deletion retains Rab18

GEF activity but lacks Rab3a GAP activity. a. AlphaFold3 prediction of Rab3GAP1 (blue) in complex with Rab3a (Rab3a in cyan with switch I, switch II and p-loop coloured in red, orange and yellow respectively). pLDDT scores are mapped by colour onto the model. PAE plot for the prediction displayed with chain A and chain B corresponding to Rab3GAP1 and Rab3a respectively. **b.** Zoomed in view of AlphaFold model of the Rab3GAP1-Rab3a interface at the

GAP domain with predicted catalytic residues (Rab3GAP1 R728 and Rab3a Q81) displayed as sticks.



Supplementary Figure 11. Comparison of previously solved Rab-GEF structures to the AlphaFold3 predicted Rab3GAP-Rab18 complex. **a.** DENND 1B and Rab35 (PDB 3TW8) **b.** RABEX-5 and Rab21 (PDB 4Q9U) **c.** SH3BP5 and Rab11a (PDB 6DJL). Each RabGEF and Rab is colored green and white, respectively, and Rabs were aligned to the position of the predicted Rab18. Rab3GAP1 in blue, Rab3GAP2N in pink and Rab18 in cyan.



Supplementary Figure 12. Cluster of basic residues on the AlphaFold2-predicted structural model C-terminal domain of Rab3GAP2. Basic patch is circled in black. Blue color represents positive charge, white for neutral, and red for negative charge.

Supplementary Table 1. Description of plasmids used in this study

Plasmid	Protein(s)	Sequence(s)	Modifications/Tags	Source
pLIB	Rab3GAP1	1-988	N-term TSP	This paper
pBIG1a	Rab3GAP1	1-988	N-term TSP	This paper
	Rab3GAP2	1-1393	C-term 3xFLAG	
pBIG1a	Rab3GAP1	1-988	N-term TSP	This paper
	Rab3GAP2(1-544)	1-544	C-term 3xFLAG	
pBIG1a	Rab3GAP1(T18P)	1-988	N-term TSP, T18P	This paper
	Rab3GAP2	1-1393	C-term 3xFLAG	
pBIG1a	Rab3GAP1(E24V)	1-988	N-term TSP, E24V	This paper
	Rab3GAP2	1-1393	C-term 3xFLAG	
pBIG1a	Rab3GAP1	1-988	N-term TSP	This paper
	Rab3GAP2(R426C)	1-1393	C-term 3xFLAG, R426C	
pOPTG	SH3BP5	1-455	N-term GST(TEV)	Jenkins et al., 2018
pOPTGcH	Rab18	1-198	N-term GST(TEV), C-term His	Jenkins et al., 2020
pOPTGcH	Rab18(R133A)	1-198	N-term GST(TEV), C-term His, R133A	This paper
pOPTGcH	Rab18(R141A)	1-198	N-term GST(TEV), C-term His, R141A	This paper
pOPTGcH	Rab18(E164A)	1-198	N-term GST(TEV), C-term His, E164A	This paper
pOPTGcH	Rab11a	1-211	N-term GST(TEV), C-term His	Jenkins et al., 2018

Supplementary Table 2. Vesicle composition used for *in vitro* GEF assays

Name	Vesicle Composition
PC – Phosphatidylcholine	95% PC, 5% 18:1 DGS-NTA(Ni), 100 nm
PC+PE – Phosphatidylethanolamine	75% PC, 20% PE, 5% 18:1 DGS-NTA(Ni), 100 nm
PC+PS – Phosphatidylserine	75% PC, 20% PS, 5% 18:1 DGS-NTA(Ni), 100 nm
AP – Autophagosome	75% PC, 10% PE, 7% PS, 3% PI 5% 18:1 DGS-NTA(Ni), 100 nm
ER - Endoplasmic Reticulum	50% PC, 25% PE, 5% PS, 15% PI 5% 18:1 DGS-NTA(Ni), 100 nm

Supplementary Table 3. Cryo-EM structural and refinement statistics

Cryo-EM data collection, refinement and validation statistics

	Human core Rab3GAP complex (EMDB-43655) (PDB 8VYB)
Data collection and processing	
Magnification	215,000x
Voltage (kV)	300
Electron exposure (e ⁻ /Å ²)	50
Defocus range (μm)	1.0-2.5
Pixel size (Å)	0.59
Symmetry imposed	C1
Initial particle images (no.)	12,427,326
Final particle images (no.)	170,636
Map resolution (Å)	3.37
FSC threshold	0.143
Map resolution range (Å)	2.2-8.2
Refinement	
Initial model used (PDB code)	AlphaFold database (Q15042) (Q9H2M9)
Model resolution (Å)	3.37
FSC threshold	0.143
Model resolution range (Å)	2.2-8.2
Map sharpening <i>B</i> factor (Å ²)	Auto-sharpening using PHENIX
Model composition	
Non-hydrogen atoms	7249
Protein residues	920
Ligands	n/a
<i>B</i> factors (Å ²)	
Protein	14.98
Ligand	n/a
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.177
Validation	
MolProbity score	0
Clashscore	0
Poor rotamers (%)	0
Ramachandran plot	
Favored (%)	95
Allowed (%)	5
Disallowed (%)	0