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Supplementary Materials for

Structural basis for FLCN RagC GAP activation in MiT-TFE substrate-selective mTORC1 regulation

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The PDF file includes:

Figs. S1 to S9
Tables S1 and S2
Legend for movie S1

Other Supplementary Material for this manuscript includes the following:

Movie S1

Supplementary Materials:

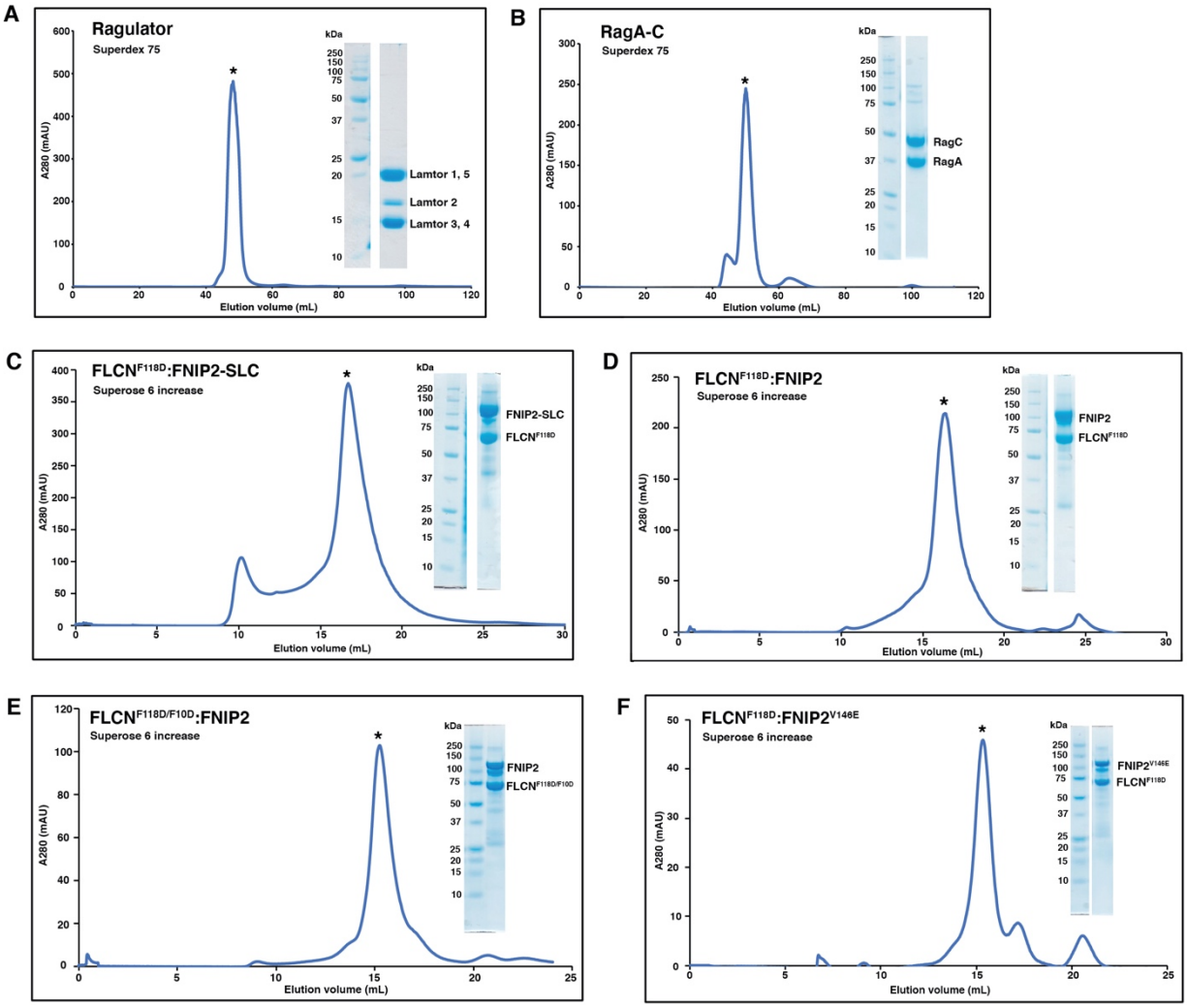
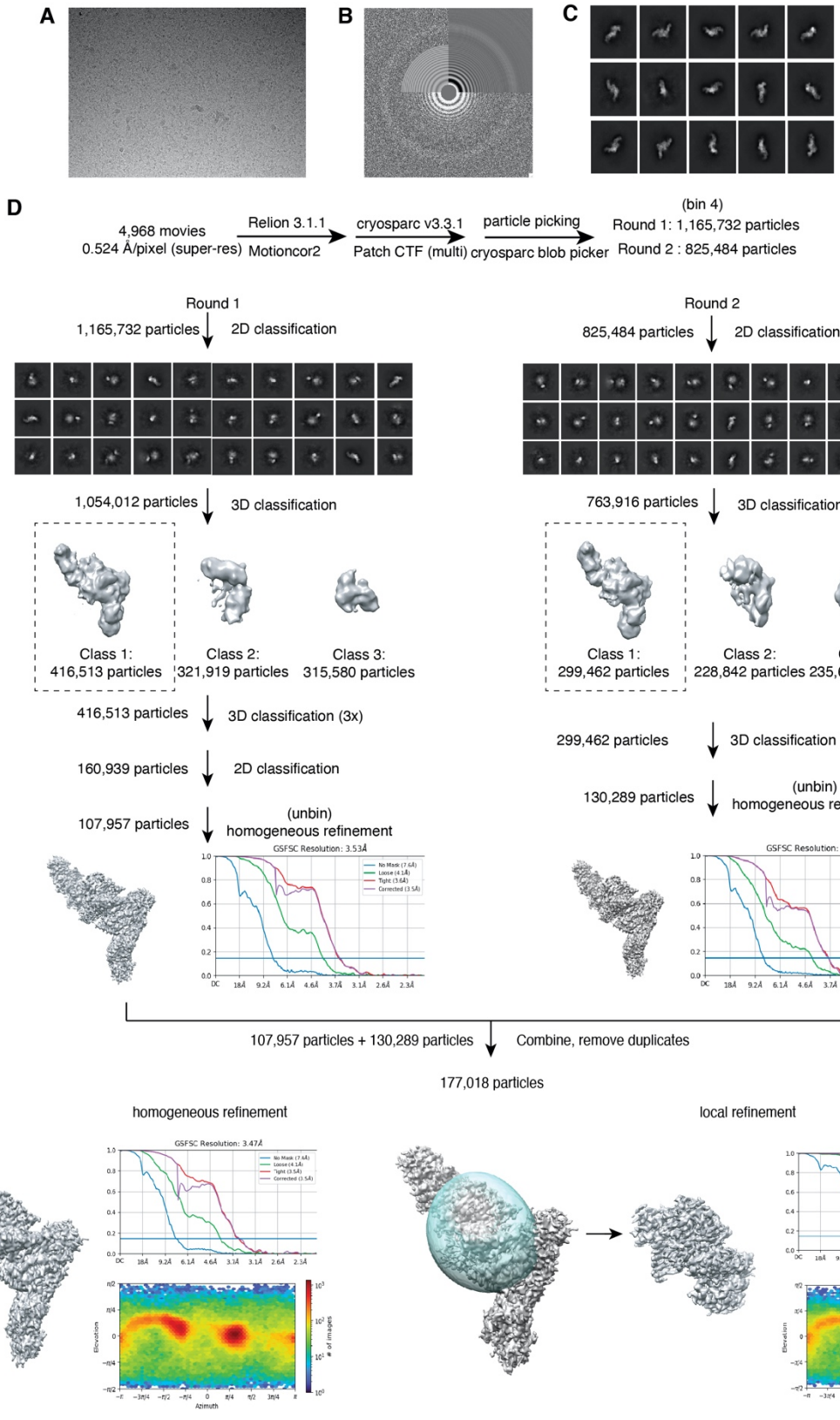


Fig. S1. Purification of individual active FLCN components. (A) Superdex 75 SEC elution profile for Ragulator and Coomassie blue stained SDS-PAGE analysis of protein sample collected from peak indicated with asterisk. (B) Superdex 75 SEC elution profile for Rag GTPases and Coomassie blue stained SDS-PAGE analysis of protein sample collected from peak indicated with asterisk. (C) Superose 6 increase SEC elution profile for FLCN^{F118D}:FNIP2-SLC fusion and Coomassie blue stained SDS-PAGE analysis of protein sample collected from peak

indicated with asterisk. (D) Superose 6 increase SEC elution profile for FLCN^{F118D}:FNIP2 and Coomassie blue stained SDS-PAGE analysis of protein sample collected from peak indicated with asterisk. (E) Superose 6 increase SEC elution profile for FLCN$^{F118D/F10D}$:FNIP2 and Coomassie blue stained SDS-PAGE analysis of protein sample collected from peak indicated with asterisk. (F) Superose 6 increase SEC elution profile for FLCN^{F118D}:FNIP2^{V146E} and Coomassie blue stained SDS-PAGE analysis of protein sample collected from peak indicated with asterisk.


A
B
C
D
4,968 movies
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Relion 3.1.1
Motioncor2
cryosparc v3.3.1
Patch CTF (multi)
particle picking
cryosparc blob picker
(bin 4)
Round 1: 1,165,732 particles
Round 2 : 825,484 particles
Round 1
1,165,732 particles
2D classification
1,054,012 particles
3D classification
Class 1:
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Class 2:
321,919 particles
Class 3:
315,580 particles
416,513 particles
3D classification (3x)
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2D classification
107,957 particles
(unbin)
homogeneous refinement
GSFSC Resolution: 3.53Å
Round 2
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2D classification
763,916 particles
3D classification
Class 1:
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Class 2:
228,842 particles
Class 3:
235,612 particles
299,462 particles
3D classification (2x)
130,289 particles
(unbin)
homogeneous refinement
GSFSC Resolution: 3.73Å
107,957 particles + 130,289 particles
Combine, remove duplicates
177,018 particles
homogeneous refinement
GSFSC Resolution: 3.47Å
Elevation
Azimuth
# of images
local refinement
GSFSC Resolution: 3.16Å

Fig. S2. Cryo-EM data processing pipeline. (A) Representative cryo-EM micrograph (B) Power spectrum and CTF estimation of micrograph shown in (A). Exemplary 2D class averages for the active FLCN complex. (D) Data processing pipeline for final map determination.

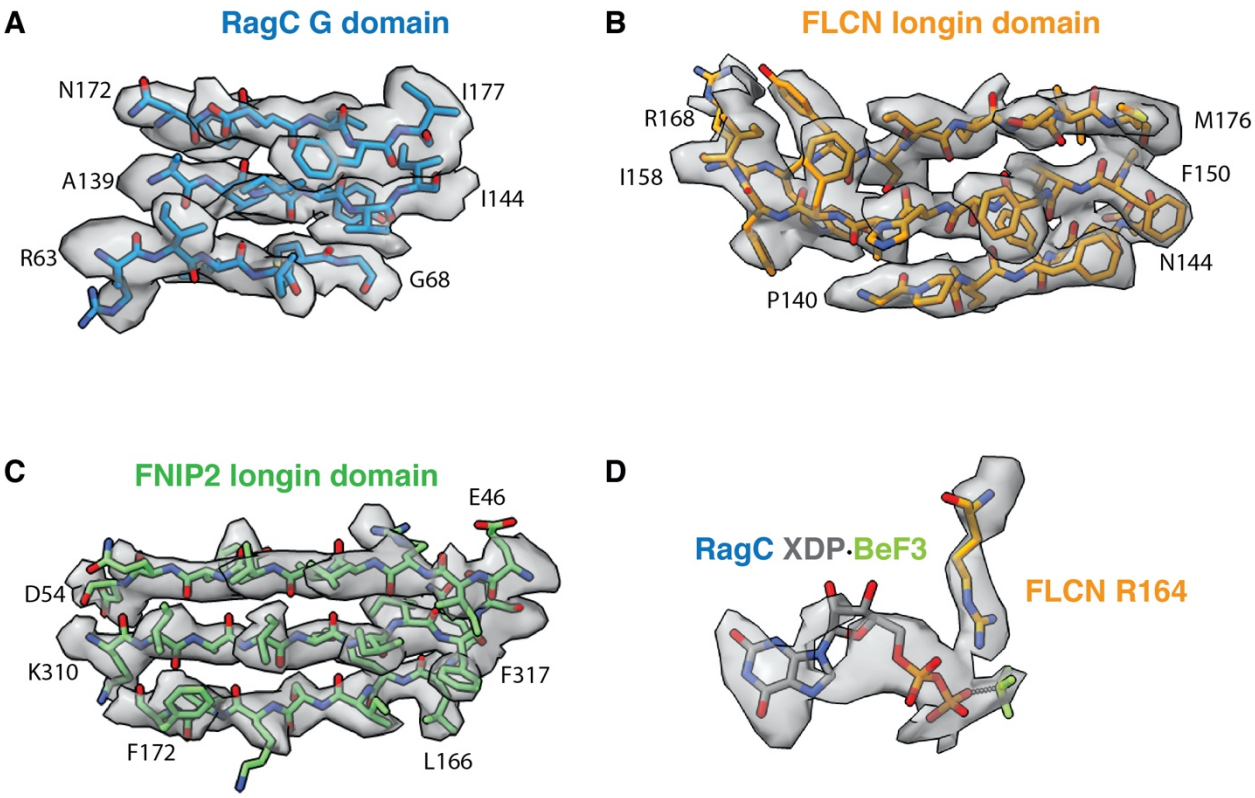

Fig. S3. AFC map-model fit. Representative refined coordinate model fit in cryo-EM density for (A) RagC (B) FLCN (C) FNIP2 (D) RagC nucleotide and FLCN arginine finger.

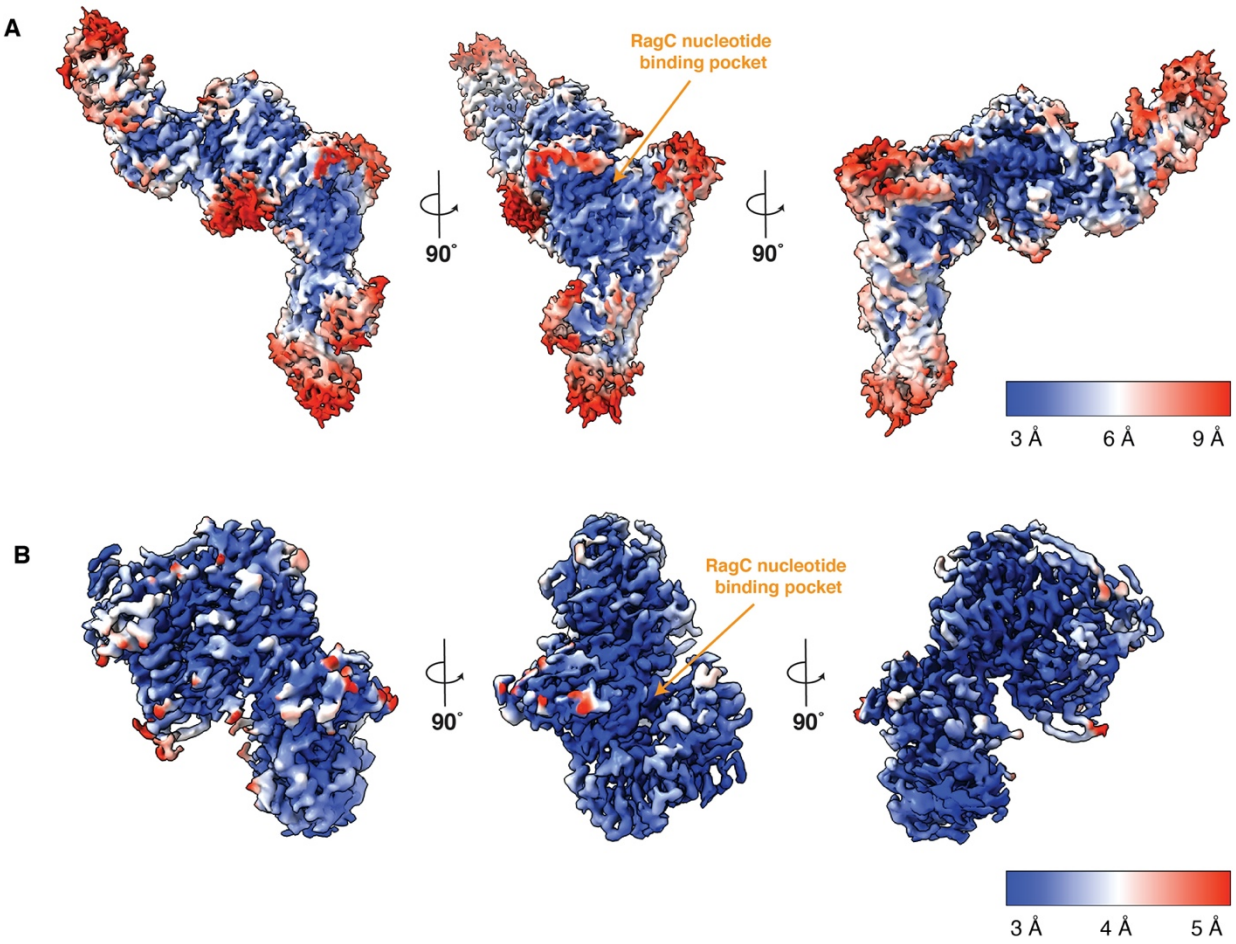

Fig. S4. AFC local resolution estimation. (A) Overall cryo-EM map for AFC colored according to local resolution. Resolution ranges from 3 Å to 9 Å. (B) Cryo-EM map of RagC – FLCN^{login}:FNIP2^{longin} interface generated from focused refinement colored according to local resolution. Resolution ranges from 3 Å to 6 Å.

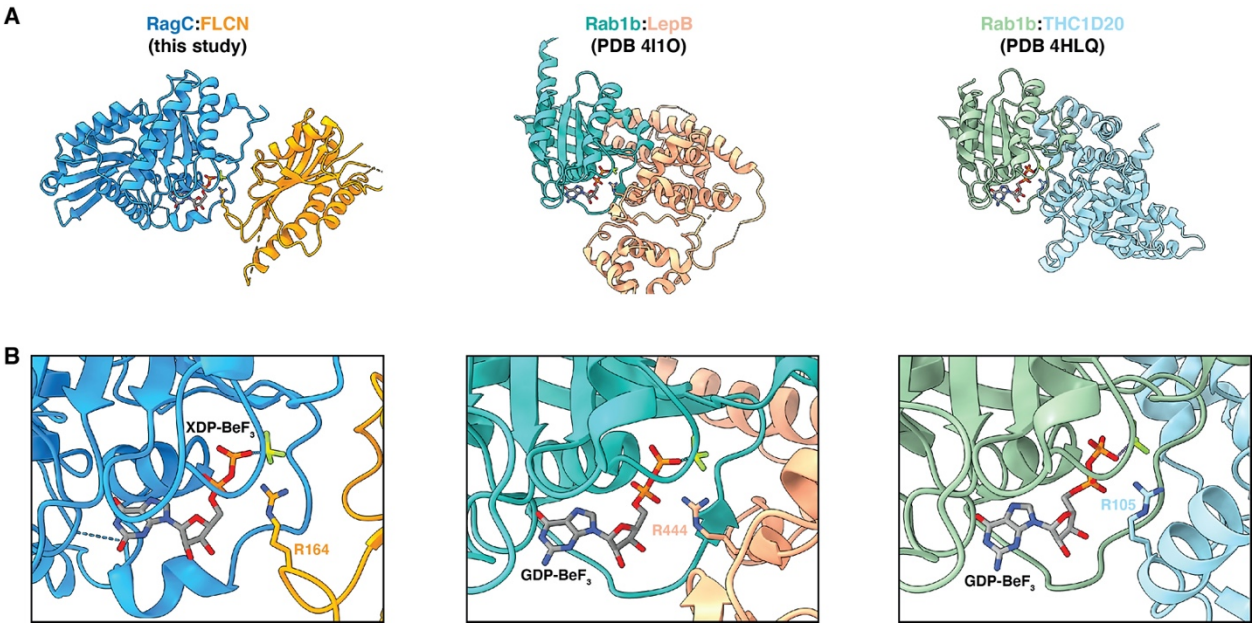

Fig. S5. Structural comparison between RagC:FLCN and other small GTPases:GAP. (A) Rab1b:LepB and Rab1b:THC1D20. (A) Structural comparison of RagC:FLCN (AFC structure), Rab1b:LepB (PDB 4I1O) and Rab1b:THC1D20 (PDB 4HLQ). Structures are shown in the same orientation. (B) Close-up of the nucleotide binding pocket with catalytic arginine-finger residue positioned in active site.

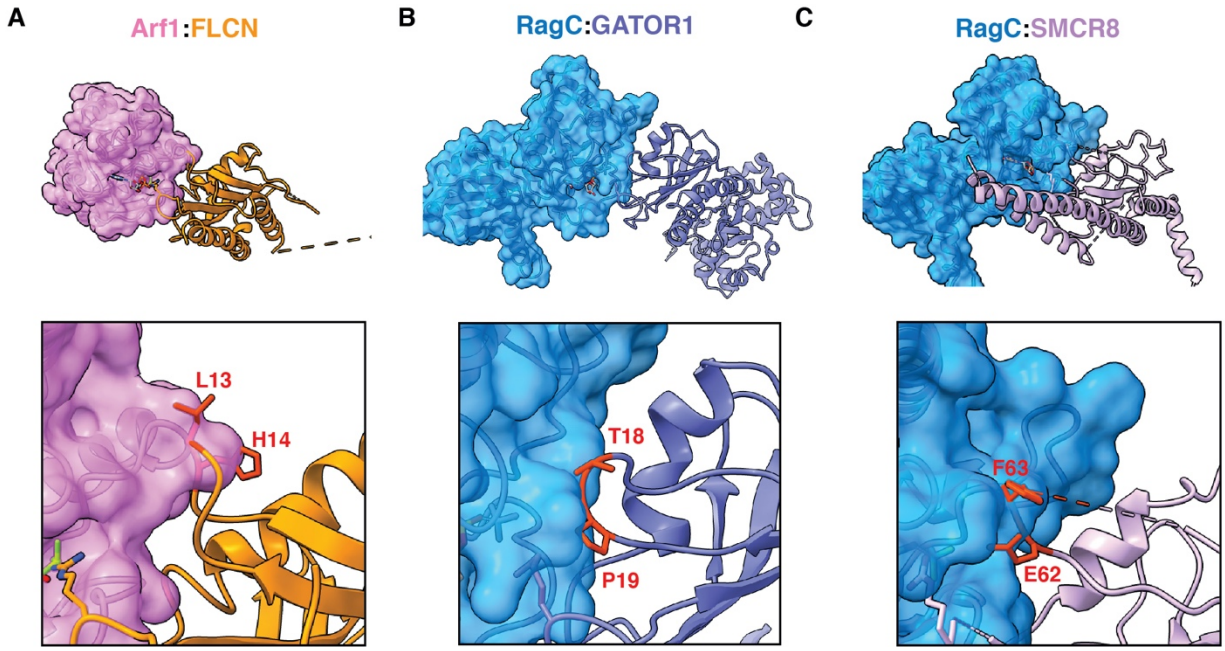

Fig. S6. Structural investigation of RagC:SMCR8 and Arf1:FLCN interface. (A) Model of Arf1 (pink) and FLCN (orange) interface generated by aligning Arf1 from Arf1:SMCR8 structure (PDB: 7MGE) with RagC in AFC structure (*27*). Residues in FLCN at interface that clash are indicated and labelled in red. (B) Model of RagC (pink) and SMCR8 (orange) interface generated by aligning SMCR8 from Arf1:SMCR8 structure (PDB: 7MGE) with FLCN in AFC structure (*27*). Residues in SMCR8 at interface that clash are indicated and labelled in red.

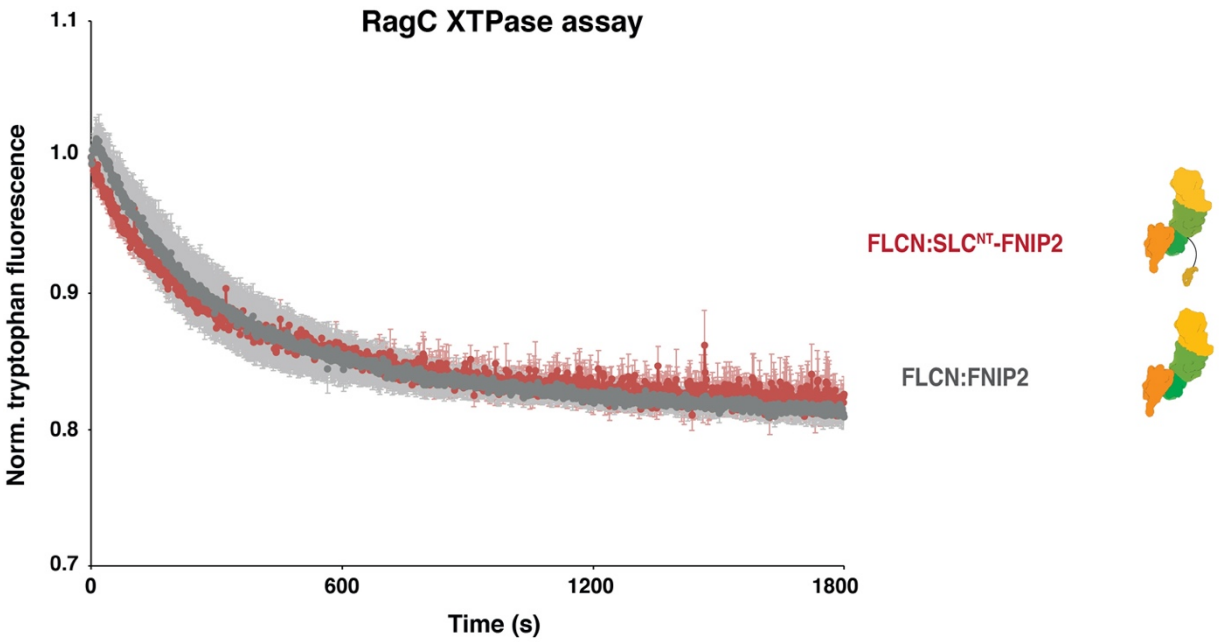

Fig. S7. GAP activity of FLCN:SLC-FNIP2. Tryptophan fluorescence XTPase assay with FLCN:SLC-FNIP2 fusion construct. $FLCN^{F118D}$: FNIP2 (gray), $FLCN^{F118D}$: SLC-FNIP2 (red), was incubated with $RagA^{GDP}$:$RagC^{XTP}$. Plotted are means ± SEM. n=3 replicates.

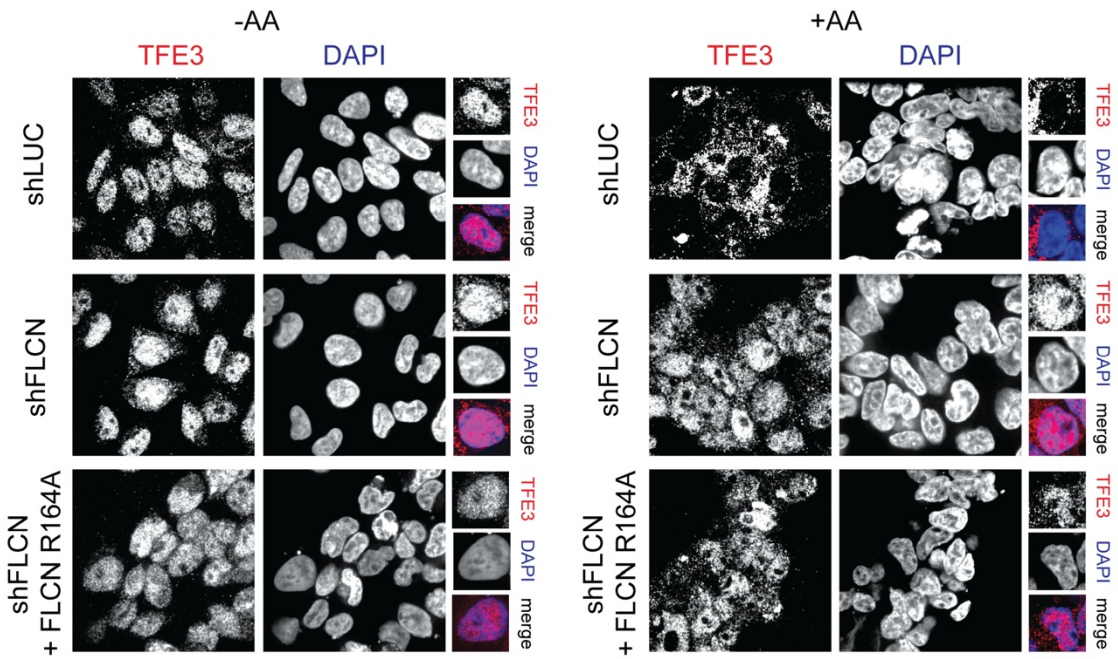

Fig. S8. Immunofluorescence for FLCN^{R164}. Immunofluorescence images of human embryonic kidney 293T (HEK293T) cells stably expressing the indicated genes shRNA and FLCN rescue constructs. Cells were starved for 2 hours for amino acids (-AA) or restimulated with complete DMEM for 2 hours (+AA).

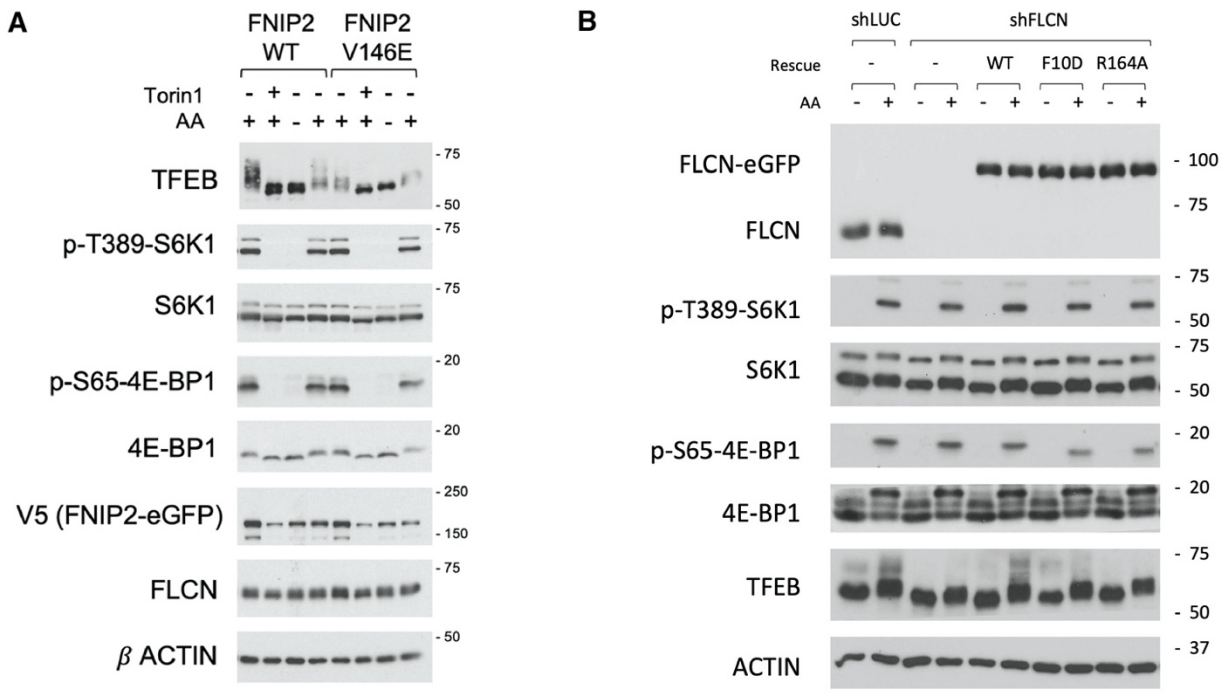

Fig. S9. Immunoblot analysis for mTORC1 targets. (A) HEK-293T cells stably expressing $FNIP2^{WT}$ or $FNIP2^{V146E}$ were either untreated or treated with Torin1, or starved for amino acids for 2 hours, or starved and then restimulated with amino acids for 15 minutes. Cells were lysed, followed by immunoblotting for the indicated proteins and phospho-proteins. (B) HEK-293T cells stably expressing shRNAs targeting the indicated genes were starved for amino acids for 2 hours, or starved and then restimulated with amino acids for 15 minutes. Cells were lysed, followed by immunoblotting for the indicated proteins and phospho-proteins.

Table S1.
Cryo-EM data acquisition and image processing.

	Active FLCN complex
Data acquisition	
Microscope	Titan Krios
Voltage (kV)	300
Camera	Quantum-K3 Summit
Magnification	165,000
Pixel size (Å)	0.524 (super-resolution)
Cumulative exposure ($e^-/Å^2$)	50
Energy filter slit width (eV)	20 eV
Defocus range (μm)	-1.0 to -2.0
Automation software	SerialEM
Exposure navigation	Image Shift
Number of movies	4968
Image processing	
Initial picked particles (no.)	Round 1: 1,165,732 Round 2: 825,484
Final refined particles (no.)	177,018
Map resolution (Å)	Overall: 3.53 Interface: 3.16
FSC threshold	0.143

Table S2.
AFC coordinate model refinement and assembly

PDB access code	8DHB
EMDB	EMD-27435
Refinement	
Software	Phenix 1.19, ISOLDE
Refinement target (Å)	3.2 (interface) 3.5 (overall)
Non-hydrogen atoms	15171
Residues	1898
Ligands	GDP (1) XDP-BeF^-_3 (1)
RagA, Ragulator, $SLC38A9^{NT}$ reference PDB	6WJ2
FLCN:FNIP2 reference PDB	6NZD
RagC reference PDB	3LLU
Map-model statistics	
R.M.S deviations	
Bond lengths (Å)	0.006
Bonds angles (Å)	1.037
Validation	
Molprobitity	1.93
Clash score	8.98
Rotamer ouliers (%)	0
Cβ outliers (%)	0
CaBLAM outliers (%)	3.95
Ramachandran	
Favored (%)	92.97
Allowed (%)	6.7
Outlier (%)	0.33
Final model composition	
Number of chains	10
Number of Residues	1898
Ligands	2
B-factors	
Protein (min/max/average)	0.27/55.15/27.92
Ligands	10.59

Movie S1. Structural rearrangement of FLCN:FNIP2 between LFC and AFC. Visualization of the inactive (LFC) and active (AFC) binding modes of FLCN:FNIP2 to Rag-Ragulator. Red spheres indicated locations of mutations FLCN F10D and FNIP2 V146E.