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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Andor iQ (Andor Technologies)
Data analysis	FRAP analysis: custom-written Matlab software. Single molecule tracking: custom-written Matlab software. Curve fitting, statistical analysis: GraphPad Prism 6. 3-D analysis: Imaris x64 v. 8.4.1

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The following figures have associated raw data that are made available in Suppl Table 1: Fig. 1-4, 6, 7; Supplementary Fig. 2, 4, 5, 6,

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For immunofluorescence and FRAP experiments, sample size (cells or spots, respectively) was selected based on commonly adopted standards in the field, resulting in statistically meaningful comparisons.
Data exclusions	No data were excluded from the experiments reported.
Replication	All experiments were carried out under standard and clearly defined conditions, and were replicated successfully by at least one researcher. One experiment (Fig. 3c) showed variable levels of FRAP suppression by GDP loading of RagB. We attribute this variability to varying levels of GDP loading efficiency of RagB. However, this result was independently verified using Rag mutants that are preferentially GDP-loaded, both in vitro and in cells, as well as by performing starvation/refeeding in cells expressing wild-type Rag GTPases.
Randomization	Randomization was applied to single molecule datasets (Fig. 4e, 4h and 6h, procedure illustrated in Supplemental Fig. 5b) which were randomly divided into 10 subsets, each including 10% of data points, then fitted and averaged to obtain average half-lives
Blinding	for co-localization analysis, the control channel was used to choose and segment the fields/cells analyzed in the experimental channel.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Research animals
☒	☐ Human research participants

Unique materials

Obtaining unique materials	we made 37 cDNA constructs that will be made publicly available via Addgene.

Antibodies

Antibodies used	see Supplementary Table 2
Validation	all antibodies were validated by the manufacturer and repeatedly validated by RNAi-mediated k.d. experiments in the literature. In some cases (i.e. RagA and RagC) we further validated the antibodies via additional knock down experiments.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK-293T, U2OS, UOK257-1, UOK257-2, SW780, and SW780-1
Authentication	HEK-293T and U2OS cells were obtained as pre-authenticated lines from the UC Berkeley Tissue Culture facility. The UOK257-1, UOK257-2, SW780, and SW780-1 were authenticated for their genotype (GATOR or FLCN deletions) by immunoblotting.
Mycoplasma contamination	All lines used were routinely tested for mycoplasma using a commercial kit (Lonza) and verified as mytoplasma-negative.

Commonly misidentified lines (See ICLAC register)	no cell lines from this register were used

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging