Supplementary Material

Nutrient Signaling and Lysosome Positioning Crosstalk Through a Multifunctional Protein, Folliculin

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                                            β1          β2
FLCN_HUMAN ss:
FLCN_HUMAN_Q8NFG4               1    M----NAIVALCHFCELHGPRTLFCTEVLHAPLPQGDGNEDSPGQGEQAEEEEGGIQMNSRMRAHSPAEG    66
FLCN_MOUSE_Q8QZS3               1    M----NAIVALCHFCELHGPRTLFCTEVLHAPLPQGAGSGDSPDQVEQAEEEEGGIQMSSRVRAHSPAEG    66
FLCN_Drosophila_Q9VS33          1    M----NAVIALCHFCEAHGPCAIFCTQTLRDTKL------------------------------------    30
FLCN_S.cerevisiae_P53237        1    MS---STVISLAHFCDKHGPRIISVTQSAEKGTL------------------------------------    31
LST7_K.lactis_QEU59399.1        1    MSVDDVGFLSLTHFCDKHGPKVLMVTQMASDLAR------------------------------------    34
Consensus_ss:                              eeeeehhhhhh    eeeeeee

                                                                                        α1
FLCN_HUMAN ss:
FLCN_HUMAN_Q8NFG4              67    ASVESSSPGPKKSDMCEGCRSLAAGHP--GYISHDKETSIKYVSHQHPSHPQLFSIVRQACVRSLSCEVC   134
FLCN_MOUSE_Q8QZS3              67    ASSESSSPGPKKSDMCEGCRSLAVGHP--GYISHDKETSIKYVSHQHPNHPQLFSIVRQACVRSLSCEVC   134
FLCN_D.melanogaster_Q9VS33     31    ---EDLSLEQQTQKTCSACNYSMGKNN-NAIYSRDTESGATFVSTKVAVLPEVASLVKQAAVLSLSNGTD    96
Lst7_S.cerevisiae_P53237       32    -GEELLVPDYPTESYCESCLLQFPEES--TRSMRCFIEDVPFITTQYS--SIRYQLLNSIIKRAFSEETM    96
Lst7_K.lactis_QEU59399.1       35    -CNELLLPEYPKDSYCESCLLRLPEPKSNATSIRSSIDDFYSVSTQYS---SIRYQLLSMIIKKTFSEET   100
Consensus_ss:                                        hhhh          ee       eeeee       h  hhhhhhhhhhhhhhhh

                                                                Longin
                                           β3        β4                  β5         α2       α3
FLCN_HUMAN ss:
FLCN_HUMAN_Q8NFG4             135    -PGREGPIFFGDEQHGFVFSHTFFIKDSLARGFQRWYSIITIMMDR--IYLINSWPFLLGKVRGIIDELQ   201
FLCN_MOUSE_Q8QZS3             135    -PGREGPIFFGDEQHGFVFSHTFFIKDSLARGFQRWYSIIAIMMDR--IYLINSWPFLLGRIRGIISELQ   201
FLCN_D.melanogaster_Q9VS33     97    ASKDGEFVFFGDSSRGHILSHTFRVSDLQARGYSQLFSIIVLMKDK--YFLLNIKPFLAEHLKKVSSELQ   164
Lst7_S.cerevisiae_P53237       97    -IYDNMPFIFFDDLRGLNLVIGFKLYDENARGNERRYCFILTVDSR--SHD-DSMKMLSEHWNFIIGGFD   162
Lst7_K.lactis_QEU59399.1      101    MSYDGSPFMFCDEHRGLNLAVGFKLEDVHARGNERRYCLILSLEKRNPCDGNDVFKTLSGNWQFIIESLS   170
Consensus_ss:                              eeeeee    eeeeeeeeee         eeeeeeee  h  hhhhhhhhhhhhhhhhhhhhhh

                                                                                          α4
FLCN_HUMAN ss:
FLCN_HUMAN_Q8NFG4             202    GKALKVFEAEQFGCPQ--------RAQRM-NTAFTPFLHQRNGNAARSLTSLTSDDNLWACLHTSFAWLL   262
FLCN_MOUSE_Q8QZS3             202    AKAFKVFEAEQFGCPQ--------RAQRM-NTAFTPFLHQRNGNAARSLTSLTSDDNLWACLHTSFAWLL   262
FLCN_Drosophila_Q9VS33        165    AAAKKTKETEEQTYSE--------RQ----RRLSGAQFLM---PTSRALLELTGEEHIFAQLHSHFSWLL   219
Lst7_S.cerevisiae_P53237      163    KMIAYIKNIHKSEFLGKNKTVENNLETLNNNAFIGSYLRANKSKFGRNLVSLTDDKFLFVRIHKWNSFLL   232
Lst7_K.lactis_QEU59399.1      171    KLIDHIKIQAREQLNR--------RQTDFAQIMGGTYLRENKQKLPVSLADIVNDQLIFLRIHKWNTFIL   232
Consensus_ss:                        hhhhhhhhhhhhhh h        hhhh                   eeehe    hhhhhhhhhhhhhh

FLCN_HUMAN ss:
FLCN_HUMAN_Q8NFG4             263    KACGSRLTEKLLEGAPTEDTLVQMEKLADLEEESESWDNSEAEEEEKAPVLPESTEGRELTQ-GPAESSS   331
FLCN_MOUSE_Q8QZS3             263    KACGSRLTEKLLEGAPTEDTLVQMEKLADLEEESESWDNSEAEEEEKAPVTPEGAEGRELTS-CPTESSF   331
FLCN_Drosophila_Q9VS33        220    LAGSRFLTEHVTFGNLPW-------------------------------LPPQSSGRPPAQRLTYNSST   257
Lst7_S.cerevisiae_P53237      233    HTV-------MNENKL------------------------------------------------------   241
LST7_K.lactis_QEU59399.1      233    TRLKSAQAETLDSDGPKD--------------------------------------------------AV   252
Consensus_ss:                        hhh hhhhhhhh

                                              α1              α2           β1         α3         α4
FLCN_HUMAN ss:
FLCN_HUMAN_Q8NFG4             332    LSGCGSWQPRKLPVFKSLRHMRQVLGAPSFRMLAWHVLMGNQVIWKSRDVDLVQSAFEVLRTMLPVGCVR   401
FLCN_MOUSE_Q8QZS3             332    LSACGSWQPPKLTGFKSLRHMRQVLGAPSFRMLAWHVLMGNQVIWKSRDVNLVHSAFEVLRTMLPVGCVR   401
FLCN_Drosophila_Q9VS33        258    LPMIESIDDPDLEEFFSLRHLKSVVRKEEFATVCYCALTGVKIVVRGD-PRKTFRFMVCLKKLLPEPMHN   326
Lst7_S.cerevisiae_P53237      242    -P--------------------------------------------------------------------   242
Lst7_K.lactis_QEU59399.1      253    KP--------------------------------------------------------------------   254
Consensus_ss:                                   hhhhhhhhhhhhhh hhhhhhhhhhhhh  eeeeee hhhhhhhhhhhhhh  hhhh

                                                                DENN
                                          α5                α6    β2                           β3
FLCN_human ss:
FLCN_HUMAN_Q8NFG4             402    IIPYSSQYEEAYRCNFLGLSPHVQIPPHVLSSEFAVIVEVHAAARSTLHPVGCEDDQSLSKYEFVVTSGS   471
FLCN_MOUSE_Q8QZS3             402    IIPYSSQYEEAYRCNFLGLSPPVPIPAHVLASEFVVVVEVHTATRSNLHPAGCEDDQSLSKYEFVVTSGS   471
FLCN_Drosophila_Q9VS33        327    LMRIDAQHQHSISSEYKIISVSNDIAVPMASSSVYRIDFLD--------------KHVNGHE--VSVKW   379
Lst7_S.cerevisiae_P53237             ----------------------------------------------------------------------
Lst7_K.lactis_QEU59399.1             ----------------------------------------------------------------------
Consensus_ss:                             hh   hhh                      eee                            eee

                                              α7                 α8                        α9
FLCN_HUMAN ss:
FLCN_HUMAN_Q8NFG4             472    PVAADRVGPTILNKIEAALTNQNLSVDVVDQCLVCLKEEWMNKVKVLFKFTKVDSRPKEDTQKLLSILGA   541
FLCN_MOUSE_Q8QZS3             472    PVAADRVGPTILNKIEAALTNQNLSVDVVDQCLICLKEEWMNKVKVLFKFTKVDSRPKEDTQKLLSVLGA   541
FLCN_Drosophila_Q9VS33        380    PGELPRKLPDLMVKLLKAVEEKNFTELVLNKQTKVLIEEWKNKVTCLNHAKST-----SVQGKLKKVLGV   444
Lst7_S.cerevisiae_P53237             ----------------------------------------------------------------------
Lst7_K.lactis_QEU59399.1             ----------------------------------------------------------------------
Consensus_ss:                                 hhhhhhhhhhhh     hhhhhhhhhhhhhh    eeeeeee           hhhhhhh
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                                                        α10
FLCN_HUMAN ss:
FLCN_HUMAN_Q8NFG4          542  SEEDNVKLLKFWMTGLSKTYKSHLMSTVRSPTASESRN  579
FLCN_MOUSE_Q8QZS3          542  SEEDNVKLLKFWMTGLSKTYKSHLMSTVRSPTATESRS  579
FLCN_Drosophila_Q9VS33     445  QPHDQ-PIINYWSTHLH---------------------  460
Lst7_S.cerevisiae_P53237        --------------------------------------
Lst7_K.lactis_QEU59399.1        --------------------------------------
Consensus_ss:                         hhhhhhhh
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Supplementary Figure 1: Alignment of multiple FLCN sequences with their respective yeast orthologue, Lst7. Multiple sequence alignment and secondary structure prediction of FLCN sequences from different species were made with PROMALS3D software (Pei, Kim, and Grishin 2008). Blue residues correspond to putative β-strands while red residues to possible α-helices. The consensus secondary structure is represented following the next code: e corresponds to β-strands and h to α-helices. Basic structural elements and domains based on the two recent human FLCN-FNIP2 structures (PDB: 6NZD, PDB: 6ULG) (Lawrence et al. 2019; Shen et al. 2019) are displayed on the top of the alignment. Protein sequences available in Uniprot (FLCN human: Q8NFG4, FLCN mouse: Q8QZS3, FLCN *D.melanogaster* Q9VS33, Lst7 S.cerevisiae P53237) and GenBank (Lst7 *K.lactis* QEU59399.1).

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FNIP2_HUMAN ss:
FNIP2_HUMAN_9P278                -----------------------------------------------------------------------
FNIP2_MOUSE_Q80TD3               -----------------------------------------------------------------------
GH23617p_D.melanogaster_Q        -----------------------------------------------------------------------
Lst4_S.cerevisiae_P34239_    1   MLGNLLRNKTSSSGFEKSSEHSDFSSVVPNVPVYCKAASTGTTKTAAGALLDTAVNVEKPSEMLSTTSPP   70
Lst4_K.lactis_Q6CXP4         1   MLGRLLRT-------------SSLSEFVPF----------GSS-----------NVTVGEEVFQNEP--   33
Consensus_ss:                      hhhh                    hhhh
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                                                                                   β1
FNIP2_HUMAN ss:
FNIP2_HUMAN_9P278            1   ----MAPTLLQKLFNKRGSSGSSAAASAQGRAPKEGP---AFSWSCSE----FDLNEIRLIVYQDC----   55
FNIP2_MOUSE_Q80TD3           1   ----MAPTLLQKLFNKRGGG----AASAQARPPKEEP---AFSWSCSE----FGLSDIRLLVYQDC----   51
GH23617p_D.melanogaster_Q    1   ----M-----------------------------------HTPPFKSNRFPFEGSQVRVLLYKE-----   25
Lst4_S.cerevisiae_P34239_   71   ILDHISDDLKLKLFGSRDI-----PYSRPIDTLQNNGGLGTDKITSIN----EKTYAFRILIIEEAGQMA  131
Lst4_K.lactis_Q6CXP4        34   --FYMTDDLKTLLYGTRDKA----LFERIANDRLH------------------NGGFRLIISQELGHVT   78
Consensus_ss:                          hhhhhheee                                     eeeeeeeehhhhhh
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                                    β2
FNIP2_HUMAN ss:
FNIP2_HUMAN_9P278           56   DRRGRQVLFDSKAVQKIEEVTAQKTEDVPIK--------ISAKCCQGSSSVSSSSSSSISSHSSSGGSSH  117
FNIP2_MOUSE_Q80TD3          52   ERRGRQVMFDSRAVQKMEEAAAQKAEDVPIK--------MSARCCQESSSSSGSSSSGSSSSHGFGGSLQ  113
GH23617p_D.melanogaster_Q   26   -DDTRRLLFDSNALQKVMHKD-QSATSASISSTSTSGGGKFLKNEKYTSLQNGKIPSKSHSSN---GSNF   90
Lst4_S.cerevisiae_P34239_  132   CRNNYRDIFDYTTSK--------------ISNSM-------------------------------------  151
Lst4_K.lactis_Q6CXP4        79   SRNNYQVVLDHSSVN---------------FHTG-------------------------------------   97
Consensus_ss:                    hh    eeeeee   hh
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Longin

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                                                  α1                 β3           β4
FNIP2_HUMAN ss:
FNIP2_HUMAN_9P278          118   HAKEQLPKYQYTRPA-SDVNMLGEMMFGS-VAM-SYKGSTLKIHYIRSPPQLMISKVFSARMGS------  178
FNIP2_MOUSE_Q80TD3         114   HAKQQLPKYQYTRPA-SDVSMLGEMMFGS-VAM-SYKGSTLKIHYIRSPPQLMISKVFSATMGS------  174
GH23617p_D.melanogaster_Q   91   IEVCAEYGYKHNRPSGADITPVGEMVFGS-LPM-SFCGTALKVHWLPEPSRILCSQVYLTPTSNGGSGHS  158
Lst4_S.cerevisiae_P34239_  152   --------------EQIRPSELKEYIFGSPVRSSD-LTQCDKIRTIPNSDLVLITRIFYYTHQ-------  199
Lst4_K.lactis_Q6CXP4        98   --------------AHIPLNELKDYIFGSSIRTIDYSASSDKIKVVKSANIVLFTRIFYLNEK-------  146
Consensus_ss:                                         hhhhhhhhh           eeeeee      eeeeeeeeee
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Disordered region

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                                                                β5           α2          α3
FNIP2_HUMAN ss:
FNIP2_HUMAN_9P278          287   STDETFSLAEETCSSNPA---MVRRKKIAISIIFSLCEKEEAQRNFQDFFFSHFPLFESHMNRLKSAIEK  353
FNIP2_MOUSE_Q80TD3         283   STDETFSLAEETCSSNPA---MVRRKKIAISIIFSLCEREAAQRDFQDFFFSHFPLFESHMNRLKGAIEK  349
GH23617p_D.melanogaster_Q  362   YRRASYCANETRSNPEMGRRQANLRRRAKLGLAVCISMSESFEEEMELFCSEHIALLESMLSRLRASTEL  431
Lst4_S.cerevisiae_P34239_  200   --------------------------YNRIAISLCIP------RILLPVVAESWSSISSWLTQTQKMLIG  237
Lst4_K.lactis_Q6CXP4       147   ------------------------STL-RIAISCCVT------DDVLPVLTECWPHISSFLDQCENTLLK  185
Consensus_ss:                     hh                         eeeeeeee       hhhhhhhhhhhhhhhhhhhhhhhhhhhhhhh
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                                                                 α4
FNIP2_HUMAN ss:
FNIP2_HUMAN_9P278          354   AMISCRK-------------IAESSLRVQ------FYVSRLMEALGEFR----GTIWNLYSVPRIAEPVW  400
FNIP2_MOUSE_Q80TD3         350   AMISCRK-------------ISESSLRVQ------FYVSRLMEALGEFR----GTIWNLYSVPRIAEPVW  396
GH23617p_D.melanogaster_Q  432   AYINHKN-----------------------------FLQIMFQAWQDTQ----QWFSDLFTAPRIKTPVW  468
Lst4_S.cerevisiae_P34239_  238   FLTKNRIMQENTGNYSNNSVIKL---SNIDIRTHYP--KEIEIMVQTLQKRVIPGLRSMSEIPRL-----  297
Lst4_K.lactis_Q6CXP4       186   YLAKND-------------TQF---LPHDWKARNC--IEVAAVLQTFQRKIIPLLSGYSDTPRL-----  231
Consensus_ss:                    hhhhh                h           hhhhhhhhhhhhhhhhhhhhhhhhhh          ee
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                                          α1                     α2
FNIP2_HUMAN ss:
FNIP2_HUMAN_9P278          401   LTMMSG-TLEKNQLCQRFLKEFTLLIEQIN---KNQFFAALLTAVLTYHLAWVPTVMPVDHPPIKAFSEK  466
FNIP2_MOUSE_Q80TD3         397   LTMMSN-TLEKNQLCQRFLKEFILLIEQVN---KNQFFAALLTAVLTYHLAWVPTVMPVDHPPIKAFSEK  462
GH23617p_D.melanogaster_Q  469   LSITTSGSKYSKTVAERFIKELCDLLSFADTKDSNFFISTMLTGILTHHLGWVATVSAFNSSGSKRSESS  538
Lst4_S.cerevisiae_P34239_  298   ---FLY-PETFKEFVHVWFKSIFNWIEIKDG-PKLGFLPLLMAMIISDYRHTIREL--------------  348
Lst4_K.lactis_Q6CXP4       232   ---FLY-PMDSIPYIKTWVKYVTNWIELKDG-PRVRFLPILLAKLRYDFASLLKEN--------------  282
Consensus_ss:                                hhhhhhhhhhhhhhhhhh        hhhhhhhhhhhhhhhhhhhh
```

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                                                                       β1          α3
FNIP2_HUMAN ss:
FNIP2_HUMAN_9P278          467   RTSQSVNMLA------KTHPYNPLWAQLGDLYGAIGSPVRLTRTVVVGKQKDLVQRILYVLTYFLRCSEL  530
FNIP2_MOUSE_Q80TD3         463   RTSQSVNMLA------KTHPYNPLWAQLGDLYGAIGSPVRLTRTVVIGKQKDLVQRILYVLTYFLRCSEL  526
GH23617p_D.melanogaster_Q  539   ASAAAIEQRAKLLQVAQKHPYNALWAQLGDLYGAIGMPPKLARTIVCGAEKLWVEKLLNVLTYFIRCSEV  608
Lst4_S.cerevisiae_P34239_  349   ---------------------------------------KTSKIVILSGNMVVANKLLFILSALLEPK--  377
Lst4_K.lactis_Q6CXP4       283   ---------------------------------------SNTRIVILTGNMNVANRLIFILTAFLGPH--  311
Consensus_ss:                     hhhhhhh                hhhhhhhhhh            eeeeeeee   hhhhhhhhhhhhhhhhhhhhh
```
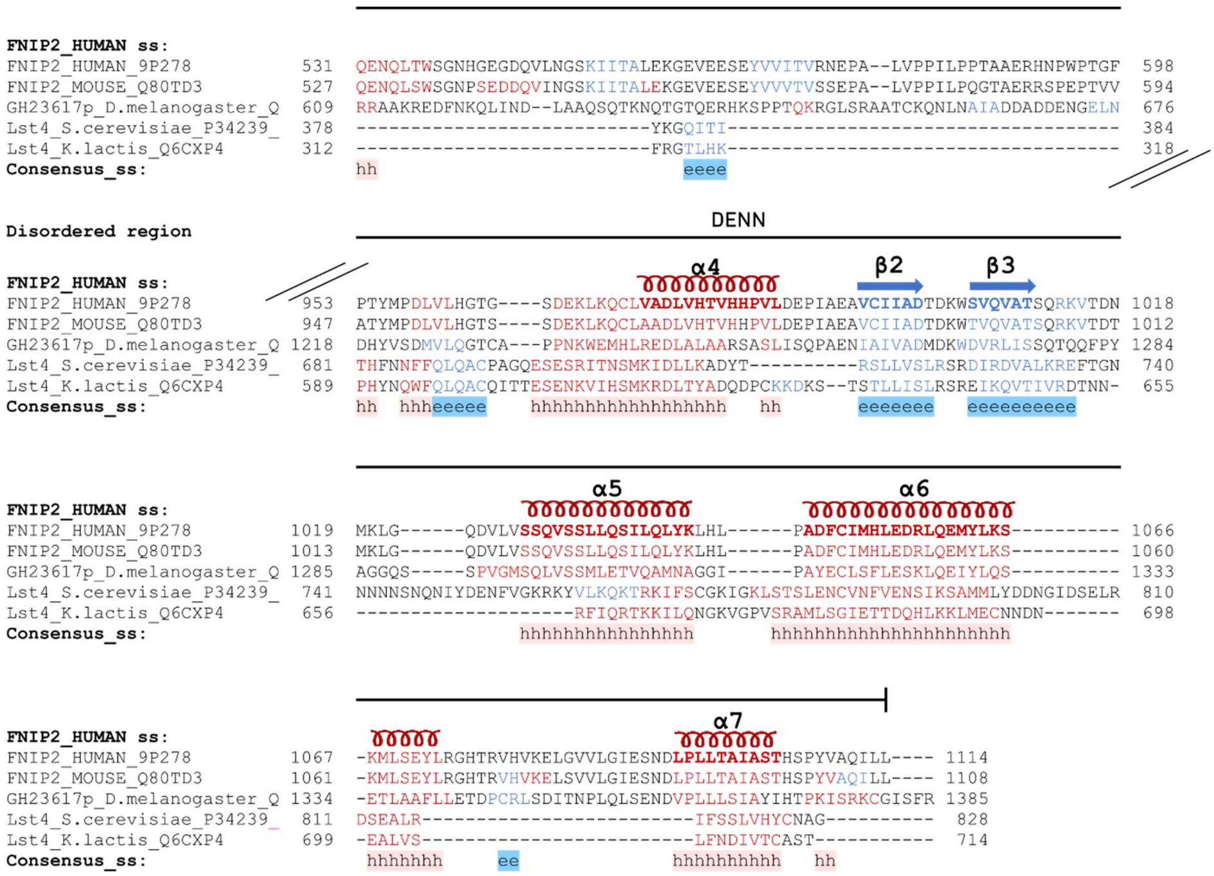
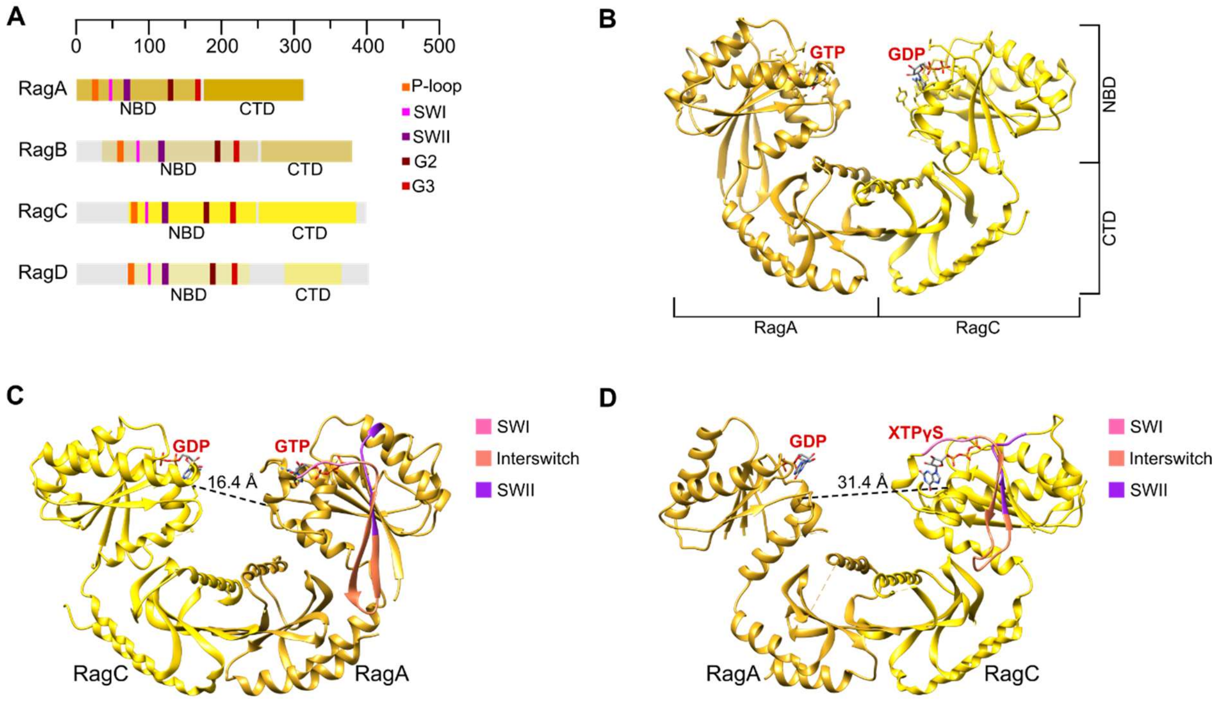



Supplementary Figure 2: Alignment of multiple FNIP2 sequences with their respective yeast orthologue, Lst4. Multiple sequence alignment and secondary structure prediction of FNIP2 sequences from different species were made with PROMALS3D software (Pei, Kim, and Grishin 2008). Blue residues correspond to putative β-strands while red residues to potential α-helices. The consensus secondary structure is represented following the next code: e corresponds to β-strands and h to α-helices. Basic structural elements and domains based on the two recent human FLCN-FNIP2 structures (PDB: 6NZD, PDB: 6ULG) (Lawrence et al. 2019; Shen et al. 2019)are displayed on the top of the alignment. Protein sequences from Uniprot (FNIP2 human: Q9P278, FNIP2 mouse: Q80TD3, GH23617p *D.melanogaster* Q8T0L7, Lst4 S.cerevisiae P34239, Lst7 *K.lactis* Q6CXP4).



Supplementary Figure 3: The Rag GTPases. (**A**) Schematic representation of the human Rag GTPases with domains coloured accordingly and typical regulatory elements from GTPases highlighted in small boxes. Top ruler represents number of amino acids. (**B**) RagA-RagC heterodimer structure with GTP and GDP bound respectively (PDB: 6U62) (Rogala et al. 2019). Both Rag GTPases are shown in cartoon representation, with RagA shown in gold and RagC in yellow. (**C**) Structure of the RagA-RagC heterodimer on its active nucleotide state (PDB: 6U62) (Rogala et al. 2019). Switch and interswitch regions from RagA-GTP are coloured according to the legend on the left. (**D**) Structure of the RagA-RagC heterodimer when it forms complex with Ragulator and FLCN-FNIP2 (PDB: 6NZD) (Lawrence et al. 2019). In this case, the Rag GTPase heterodimer is on its inactive nucleotide binding state, that is RagA-GDP and RagC-GTP (XTPγS). Switch and interswitch regions from RagC-GTP are coloured according to the legend on the right.

References

Lawrence, Rosalie E., Simon A. Fromm, Yangxue Fu, Adam L. Yokom, Do Jin Kim, Ashley M. Thelen, Lindsey N. Young, et al. 2019. "Structural Mechanism of a Rag GTPase Activation Checkpoint by the Lysosomal Folliculin Complex." *Science*, October, eaax0364. https://doi.org/10.1126/science.aax0364.

Pei, Jimin, Bong-Hyun Kim, and Nick V. Grishin. 2008. "PROMALS3D: A Tool for Multiple Protein Sequence and Structure Alignments." *Nucleic Acids Research* 36 (7): 2295–2300. https://doi.org/10.1093/nar/gkn072.

Rogala, Kacper B., Xin Gu, Jibril F. Kedir, Monther Abu-Remaileh, Laura F. Bianchi, Alexia M. S. Bottino, Rikke Dueholm, et al. 2019. "Structural Basis for the Docking of MTORC1 on the Lysosomal Surface." *Science*, October, eaay0166. https://doi.org/10.1126/science.aay0166.

Shen, Kuang, Kacper B. Rogala, Hui-Ting Chou, Rick K. Huang, Zhiheng Yu, and David M. Sabatini. 2019. "Cryo-EM Structure of the Human FLCN-FNIP2-Rag-Ragulator Complex." *Cell* 179 (November): 1–11. https://doi.org/10.1016/j.cell.2019.10.036.