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Supplemental Material to:

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FLCN, a novel autophagy component, interacts with GABARAP and is regulated by ULK1 phosphorylation

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Supp Figure 1

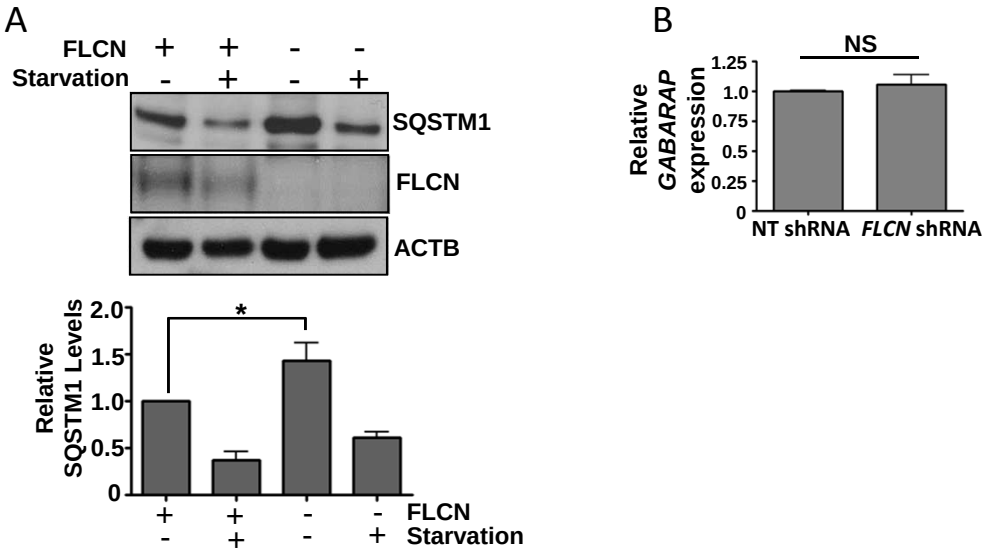

Fig. S1: Autophagy is impaired in *Flcn*-deficient MEF cells

(A) FLCN-expressing or deficient MEF cells were given normal growth media or starved in KRB for 4 h. Total cell lysates were analysed for SQSTM1 levels by western blotting. Relative levels were determined by densitometry and are plotted as mean ± S.E.M. for three independent experiments. * $p < 0.05$ (B) *GABARAP* mRNA expression levels in HK2 cells expressing and deficient for *FLCN* were determined by Q-PCR.

Supp Figure 2

A

MNAIVALCHFCELHGPRTLFCTEVLHAPLPQGDGNEDSPGQGEQAEEEEGGIQMNSRMR**.AHSPAEGASVESS**
SPGPK.KSDMCEGCR.**SLAAGHPGYISHDKETSIK**.YVSHQHPSHPQLFSIVRQACVRSLSCEVCPGREGPIFFGD
EQHGFVFSHTFFIKDSLARGFQRWYSIITIMMDRIYLINSWPFLLGKVRGIIDELQGKALKVFEAEQFGCPQRAQR
MNTAFTPFLHQRNGNAARSLTSLTSDDNLWACLHTSFAWLLKACGSRLTEK**.LLEGAPTEDTLVQMEK**.LADLEE
ESESWDNSEAEEEEKAPVLPESTEGR.ELTQGPAESSSLSGCGSWQPRKLPVFKSLRHMRQVLGAPSFR.MLA
WHVLMGNQVIWK.SRDVDLVQSAFEVLRTMLPVGCVRIIPYSSQYEEAYRCNFLGLSPHVQIPPHVLSSEFAVIVE
VHAAARSTLHPVGCEDDQSLSKYEFVVTSGSPVAADRVGPTILNKIEAALTNQNLSVDVVDQCLVCLKEEWMNK
VKVLFKFTKVDSRPKEDTQKLLSILGASEEDNVKLLKFWMTGLSKTYKSHLMSTVRSPTASESRN

B

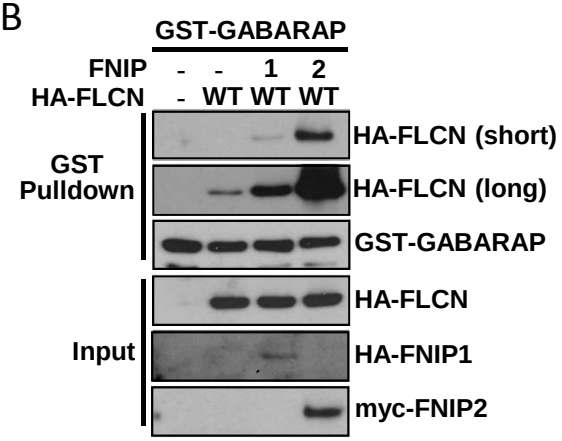

Fig. S2: The FLCN/FNIP complex binds to GABARAP

(A) The peptide sequence of the longest isoform of human FLCN is shown. Highlighted are the peptides identified in two technical replicate LC-MS/MS analyses of NTAP-GABARAP immunoprecipitates (first replicate is bold, second replicate is underlined). (B) Bacterially expressed GST-GABARAP was used as bait for lysates containing HA-FLCN with or without FNIP1 or FNIP2, where indicated. Following GST purification, bound HA-FLCN was detected by western blot.

Supp Figure 3

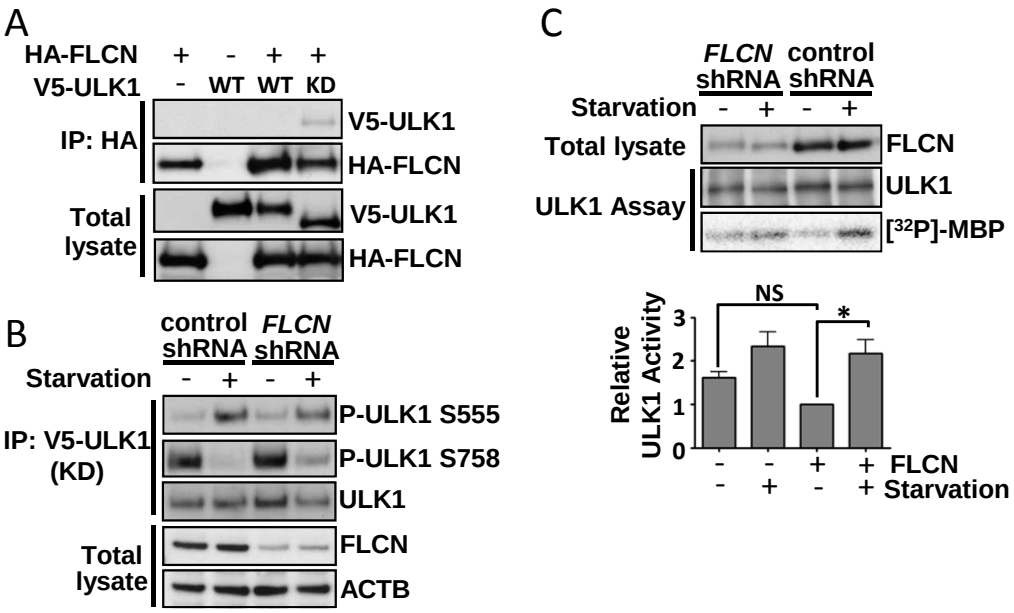

Fig. S3: FLCN interacts with ULK1 but does not alter ULK1 activity

(A) HA-FLCN was co-expressed with V5-tagged wild-type (WT) or kinase dead (KD) ULK1 as indicated in HEK293 cells, and subjected to HA immunoprecipitation. ULK1 bound to FLCN was detected by western blotting. (B) Control HK2 cells and those with stable knockdown of *FLCN* were transfected with kinase dead ULK1 for 24 h, followed by starvation for 4 h in KRB where indicated. V5-tagged ULK1 was immunoprecipitated and probed for phosphorylation at Ser555 and Ser758. (C) Endogenous ULK1 activity was measured by incorporation of [^{32}P] into myelin basic protein (MBP). The graph shows relative ULK1 activity across three independent experiments, mean ± S.E.M. NS = not significant, * $p < 0.05$.

Supp Figure 4

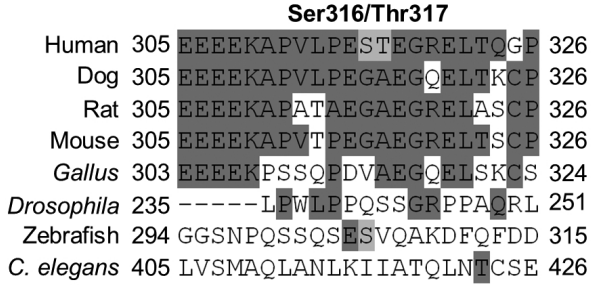

Fig. S4: Ser316/Thr317 are poorly conserved

A multi-species alignment of FLCN proteins using Clustal Omega shows that the potential ULK1-mediated phosphorylation sites, Ser316 and Thr317 are not well conserved.

Supp Figure 5

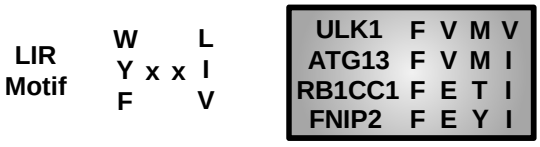

Fig. S5: FNIP2 contains a potential LIR motif
The canonical LIR motif together with a sequence comparison of LIR motifs identified in ULK1, ATG13 and RB1CC1, as well as a potential LIR motif within FNIP2.