Supplemental materials

Supplemental tables:

Table S1: Oligonucleotide sequences used within the study.

Table S2: Split-luciferase complementation based protein-protein assay results.

Table S3: Summary of studied genes and protein localization data.

Table S4: Mass spectrometry results of CG9186 binding partners.

Supplemental figure legends:

Fig. S1: Localization of the murine ATGL protein in Drosophila Kc167 tissue culture cells. The protein was expressed as a GFP-fusion protein in tissue culture cells in the absence of OA. 4 hours before fixation and staining of the cells, LD induction was started by the addition of 1000 µM OA. Cells were counterstained for LDs using the hydrophobic BODIPY493/503 stain. Images show maximum intensity projections of z-stacks recorded by confocal microscopy. Note that in cells with high GFP expression, no LDs are detectable. On cells with lower GFP expression, the fusion protein localizes to LDs. Scale bar represents 5 µm.

Fig. S2: Identification of CG9186 candidate binding partners by co-immunoprecipitation. The table provides the number of peptide spectrum matches for four candidate CG9186 interactors identified by the co-immunoprecipitation and mass spectrometry (cf. Fig. 4). The co-immunoprecipitations were performed either in the absence (“no OA” columns) or the presence (“with OA” columns) of 400 µM oleic acid using either GFP alone or GFP-tagged CG9186 as a bait.

Fig. S3: Subcellular localization of truncated Jabba protein variants. The indicated GFP-tagged Jabba isoforms were expressed in Drosophila Kc167 cells in the presence of 400 µM OA for 3 days. Cells were fixed and stained with LipidTOX HCS Deep Red and imaged by confocal microscopy. Images show a single focal plane. Scale bars represent 5 μm.

Fig. S4: Primary sequence analysis of the Jabba protein. (A) Alignment of all currently known Jabba protein isoforms. The orange and green bars mark the most hydrophobic (orange) and negatively charged (green) amino acid stretches, which were deleted in the functional experiments shown in Figs. 6 and 7. (B) Kyte and Doolittle hydrophobicity plot of the Jabba isoform B done with the ProtParam tool (1) using standard settings. The orange bar represents the hydrophobic amino acids highlighted in (A). (C) Representation of positively and negatively charged amino acid residues of the Jabba protein isoform B as computed by the PepInfo tool (2) using the standard settings. The green bar marks the position of the charged amino acids 228 to 243 highlighted in (A). The light blue bar marks the greater stretch of negatively charged amino acids 225 to 268.

Fig. S5: Localization of the Ubiquitin protein in Drosophila Kc167 tissue culture cells and interaction test results of a mutated CG9186 protein variant. (A) Ubiquitin was fused to GFP and expressed in the presence of 400 μM OA. Cells were fixed and counterstained for LDs using the hydrophobic BODIPY493/503 stain. Images show a single focal plane recorded by confocal microscopy. (B) Interaction test result matrices of combinations between a mutated variant of CG9186 where all lysine residues were replaced by arginine in order to prevent ubiquitination. A color-code for the different interaction scores is provided. Scale bar represents 5 μm.

Fig. S6: Primary amino acid sequence of CG9186 with selected sequence features. The light blue box marks the LD-targeting sequence spanning amino acids 141-200. The grey box demarcates the C-terminal amino acids 200-307, which are necessary for the induction of LD clusters. The orange rectangles highlight the catalytical triad spanning active site serine at position 119 (within the sequence GHSIG) together with Aspartate 254 (D) and Histidine 289 (H).

All lysine (K) residues are highlighted by bold face and bigger font size. Ubiquitination of the conserved lysine residues at positions 271 and 280 (highlighted in red) could be confirmed by mass spec analysis (Fig. 8). Lysine residues marked by three asterisks are conserved in *Drosophila melanogaster*, *Culex quinquefaciens*, *Aedes aegypty*, *Anopheles gambiae*, *Mus musculus*, and *Homo sapiens*. The lysine residue marked by two asterisks is conserved in all species apart from humans. Lysine residues marked by one asterisk are only conserved in the invertebrate species.

Querying the iUbiq-Lys (3, 4) and Ubpred (5) services with the CG9186 sequence did not result in any significant ubiquitination predictions (data not shown). The BDM-PUB (6) tool predicted ubiquitination of lysines 210, 213, 219, and 271; whereas the tool RUBI (7) only predicted ubiquitination of lysine 105.

Supplemental references:

1. Wilkins, M. R., Gasteiger, E., Bairoch, A., Sanchez, J. C., Williams, K. L., Appel, R. D., and Hochstrasser, D. F. (1999) Protein identification and analysis tools in the ExPASy server. *Methods Mol. Biol.* 112, 531–52
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3. Qiu, W.-R. R., Xiao, X., Lin, W.-Z. Z., and Chou, K.-C. C. (2015) iUbiq-Lys: prediction of lysine ubiquitination sites in proteins by extracting sequence evolution information via a gray system model. *J. Biomol. Struct. Dyn.* 33, 1731–42

4. Wang, P., Xiao, X., and Chou, K.-C. C. (2011) NR-2L: a two-level predictor for identifying nuclear receptor subfamilies based on sequence-derived features. *PLoS ONE* 6, e23505

5. Radivojac, P., Vacic, V., Haynes, C., Cocklin, R. R., Mohan, A., Heyen, J. W., Goebl, M. G., and Iakoucheva, L. M. (2010) Identification, analysis, and prediction of protein ubiquitination sites. *Proteins* 78, 365–80

6. Li, A., Gao, X., Ren, J., Jin, C., and Xue, Y. (2009) BDM-PUB: computational prediction of protein ubiquitination sites with a Bayesian discriminant method.

7. Walsh, I., Di Domenico, T., and Tosatto, S. C. (2014) RUBI: rapid proteomic-scale prediction of lysine ubiquitination and factors influencing predictor performance. *Amino Acids* 46, 853–62

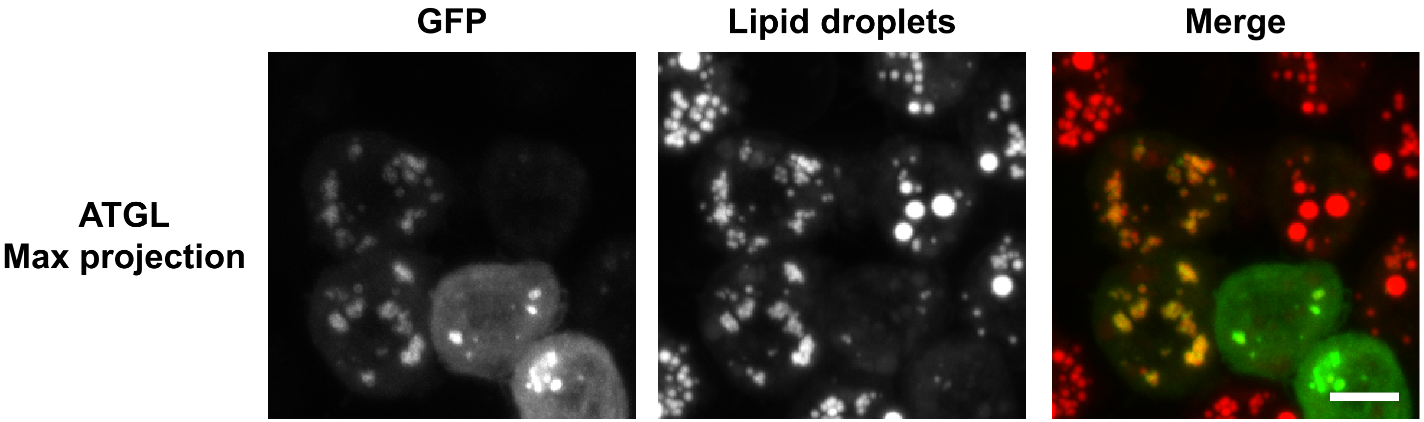
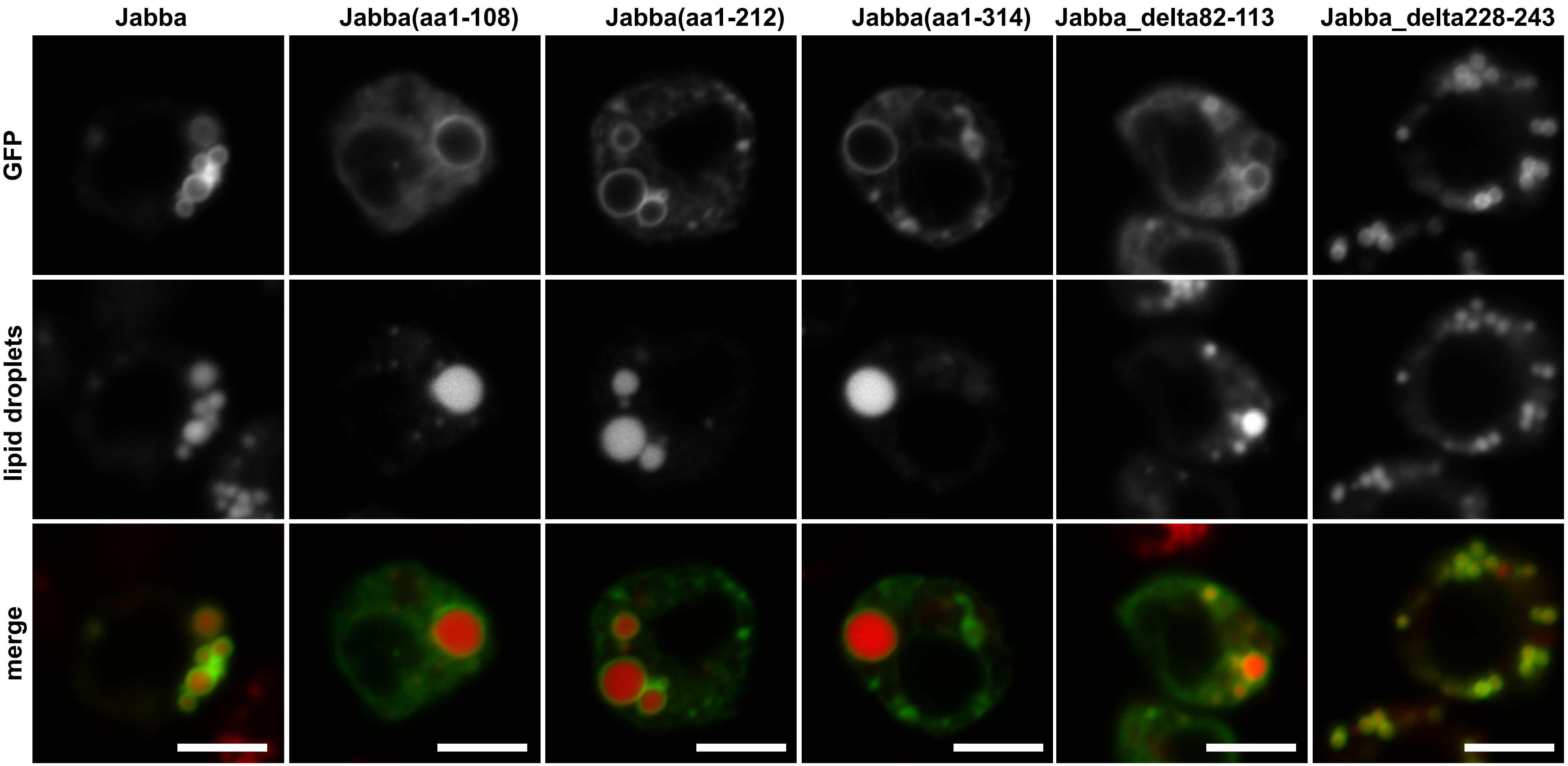


Fig. S1

Protein	Count CG9186 no OA	Count GFP no OA		Count CG9186 with OA	Count GFP with OA
Hmu	7	0		6	1
Cpr	5	1		2	1
Pvr	5	0		2	0
Gp93	11	1		9	4

Fig. S2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Jabba(aa1-314)
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Jabba_delta228-243
GFP
lipid droplets
merge

Fig. S3

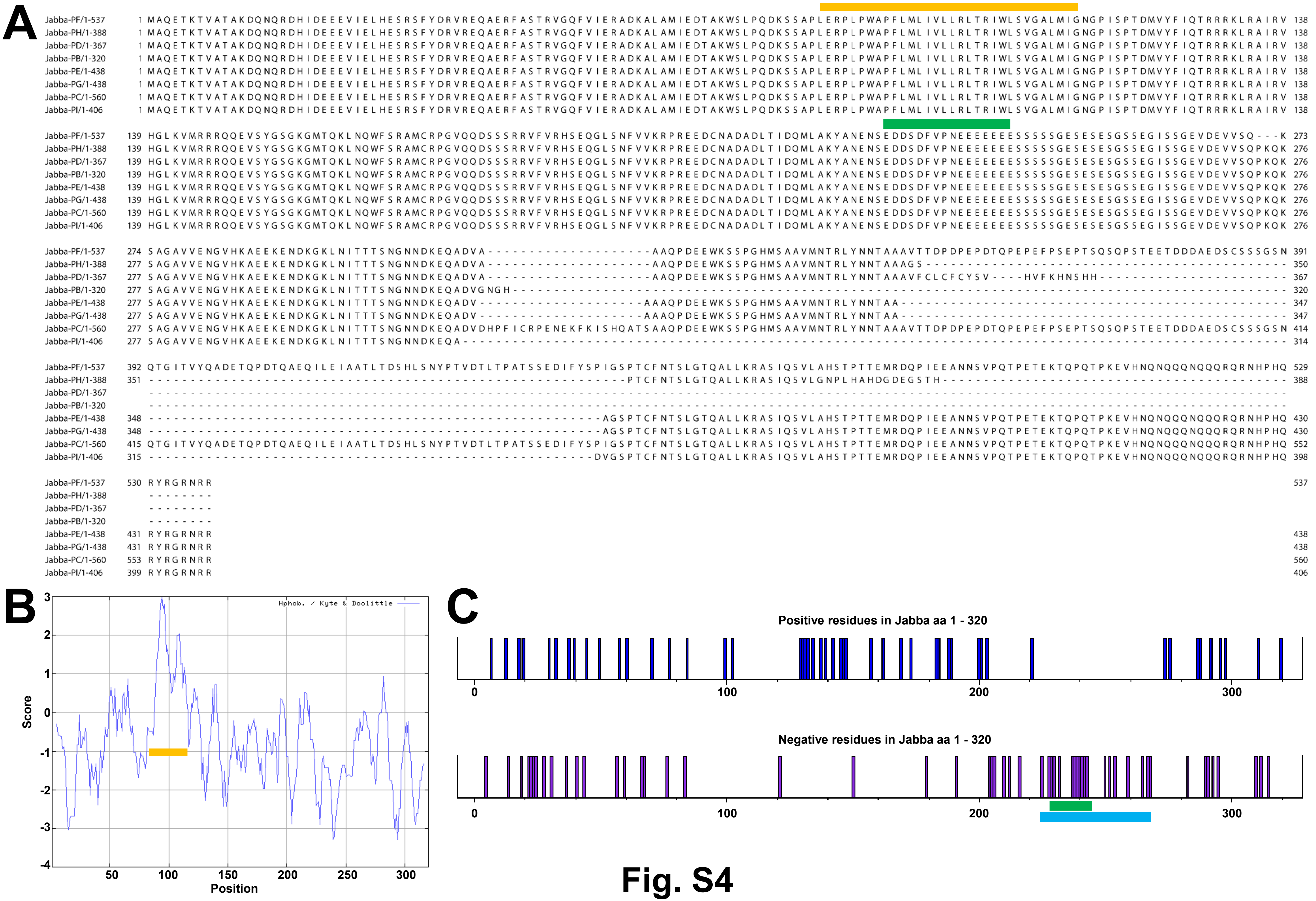

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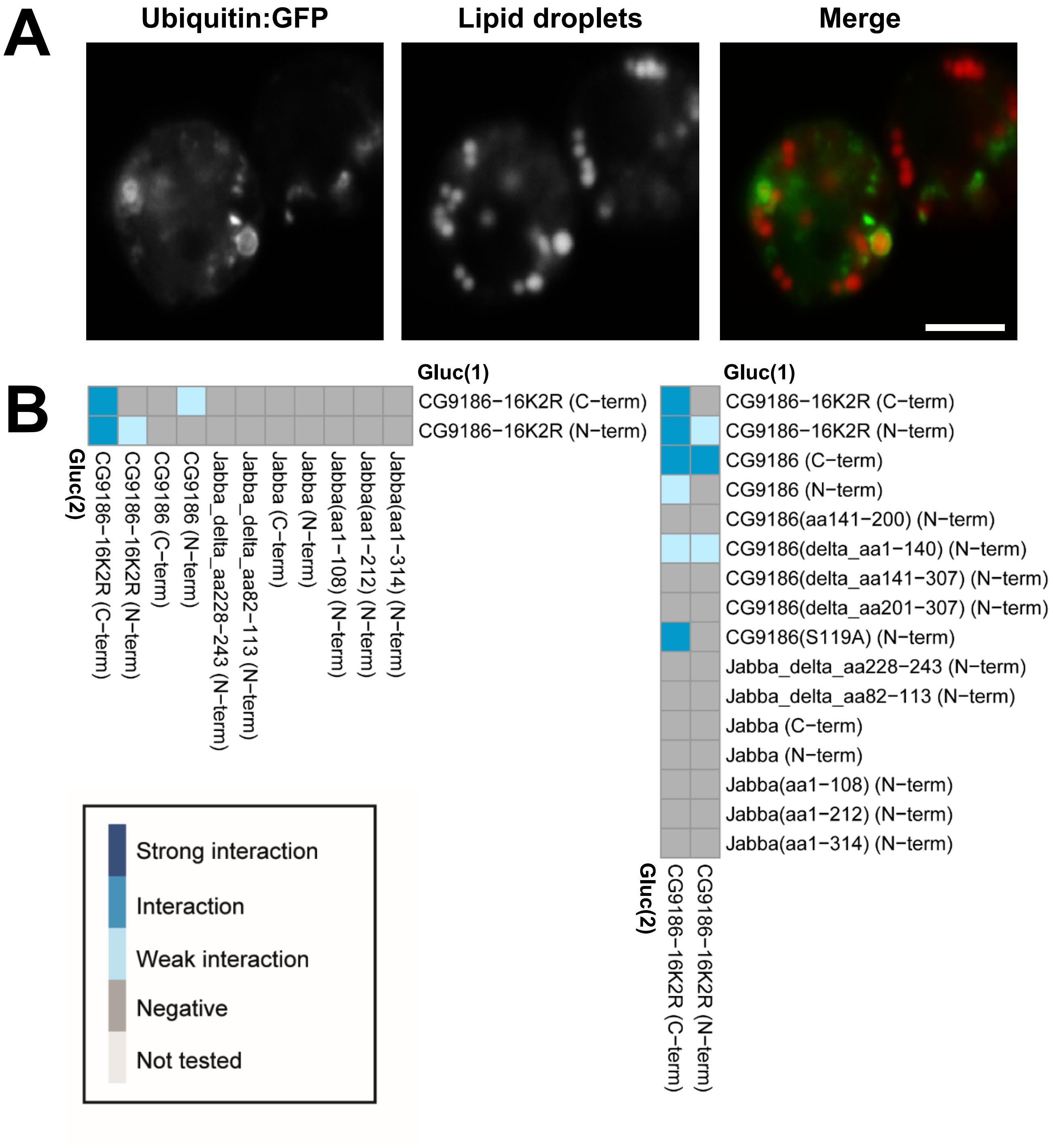
Fig. S4



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CG9186−16K2R (C−term)	▓	░	░	▒	░	░	░	░	░	░	░
CG9186−16K2R (N−term)	▓	▒	░	░	░	░	░	░	░	░	░

Gluc(1) \ Gluc(2)	CG9186−16K2R (C−term)	CG9186−16K2R (N−term)
CG9186−16K2R (C−term)	▓	░
CG9186−16K2R (N−term)	▓	▒
CG9186 (C−term)	▓	▓
CG9186 (N−term)	▒	░
CG9186(aa141−200) (N−term)	░	░
CG9186(delta_aa1−140) (N−term)	▒	▒
CG9186(delta_aa141−307) (N−term)	░	░
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CG9186(S119A) (N−term)	▓	░
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Jabba_delta_aa82−113 (N−term)	░	░
Jabba (C−term)	░	░
Jabba (N−term)	░	░
Jabba(aa1−108) (N−term)	░	░
Jabba(aa1−212) (N−term)	░	░
Jabba(aa1−314) (N−term)	░	░

█ Strong interaction
▓ Interaction
▒ Weak interaction
░ Negative
□ Not tested

Fig. S5

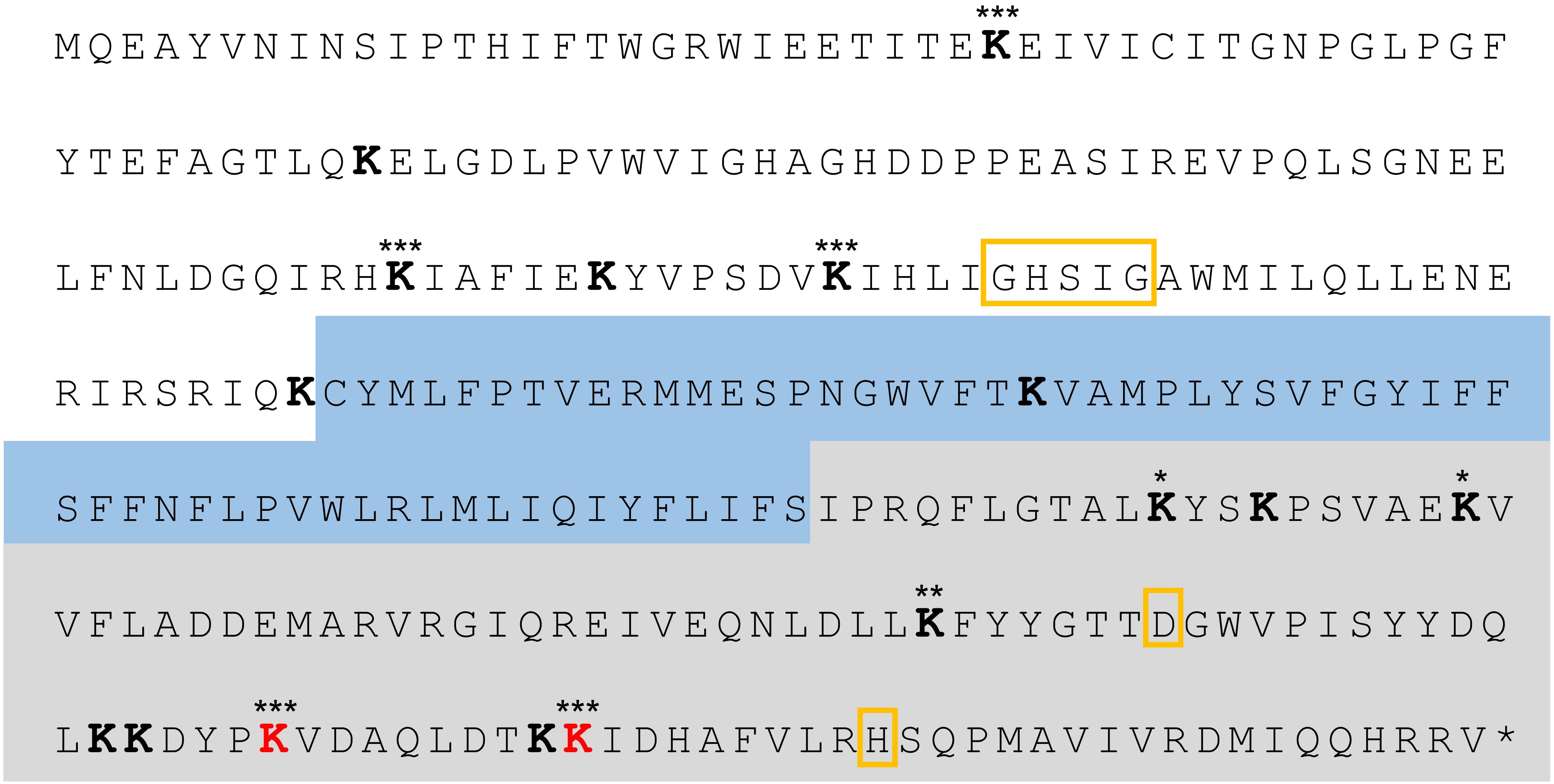

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Fig. S6