

# **A tunable LIC1-adaptor interaction modulates dynein activity in a cargo-specific manner**

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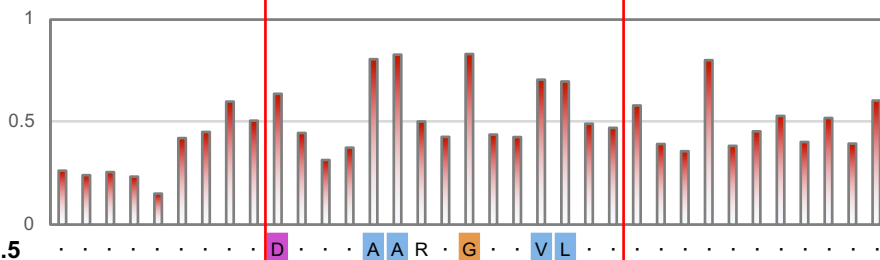
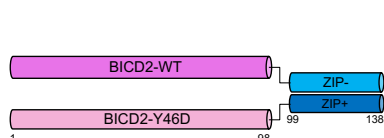
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**a****CC1-box containing adaptors**

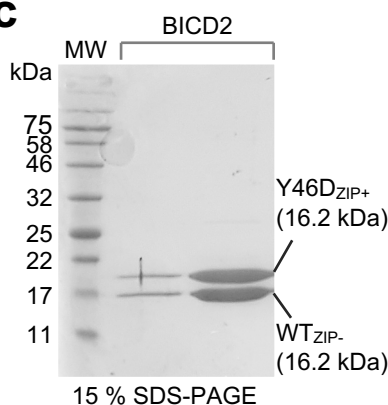
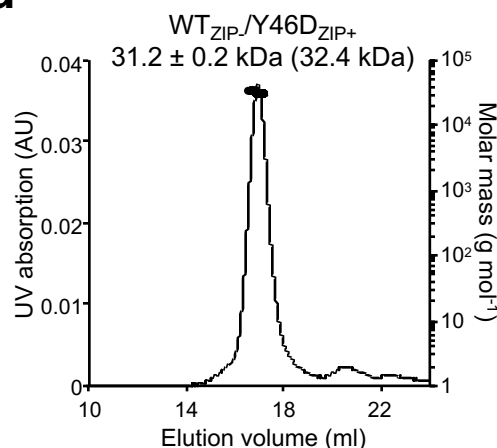
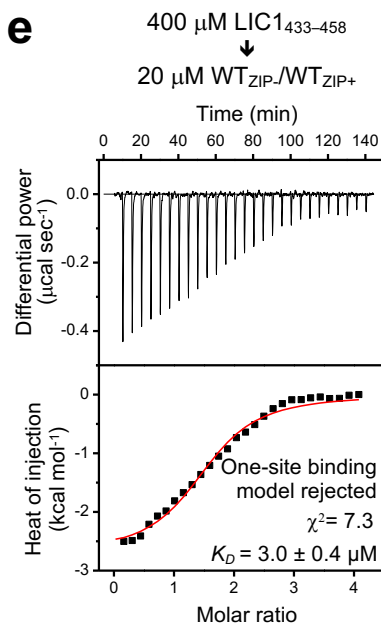
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|-----------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BICD2 Q8TD16/30–64    | S | H | E | L | A | E | T | T | R | E | K | I | Q | A | A | E | Y | G | L | A | V | L | E | E | K | H | Q | L | K | L | Q | F | E | E | L |
| BICD1 Q96G01/21–55    | T | K | E | L | T | E | T | T | H | E | K | I | Q | A | A | E | Y | G | L | V | V | L | E | E | K | L | T | L | K | Q | Q | Y | D | E | L |
| BICDL1 Q6ZP65/100–134 | L | S | V | I | R | K | E | K | D | L | V | L | A | A | R | L | G | K | A | L | L | E | R | N | Q | D | M | S | R | Q | Y | E | Q | M |   |
| BICDL2 A1A5D9/51–85   | A | L | Q | L | Q | K | E | K | D | L | L | L | A | A | E | L | G | K | M | L | L | E | R | N | E | E | L | R | R | Q | L | E | T | L |   |
| HAP1 P54257/194–228   | Y | G | M | V | L | Q | R | E | R | D | L | N | T | A | A | R | I | G | S | L | V | K | Q | N | S | V | L | M | E | E | N | S | K | L |   |
| TRAK1 Q9UPV9/116–150  | T | R | L | L | E | E | K | E | R | D | L | E | L | A | A | R | I | G | S | L | L | K | K | N | K | T | L | T | E | R | N | E | L | L |   |
| TRAK2 O60296/116–150  | T | H | L | L | A | E | R | D | R | D | L | E | L | A | A | R | I | G | A | L | L | K | R | N | H | V | L | S | E | Q | N | E | S | L |   |
| Spindly Q96EA4/10–44  | R | C | R | L | K | E | A | E | E | E | R | L | K | A | A | Q | Y | G | L | Q | L | V | E | S | Q | N | E | L | Q | N | Q | L | D | K |   |

**Conservation scores**

(from 100 CC1-box-containing adaptors)

**Conservation score  $\geq 0.5$** **b**

|                            |   |      |   |   |   |   |   |   |   |   |   |   |   |   |   |    |    |                          |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |     |   |   |   |
|----------------------------|---|------|---|---|---|---|---|---|---|---|---|---|---|---|---|----|----|--------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|---|---|---|
|                            | 1 | BICD |   |   |   |   |   |   |   |   |   |   |   |   |   | 98 | 99 | ZIP+/ZIP- leucine zipper |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   | 138 |   |   |   |
| BICD2-WT <sub>ZIP-</sub>   | M | S    | A | P | . | . | . | . | D | G | E | S | L | E | I | R  | A  | A                        | F | L | R | Q | N | T | A | L | R | T | E | V | A | E | L | E | Q | V | R | L | E | N | E | V | S | Q | Y | E   | T | R | Y |
| BICD2-Y46D <sub>ZIP+</sub> | M | S    | A | P | . | . | . | . | D | G | E | S | L | E | I | E  | A  | A                        | F | L | R | Q | N | T | A | L | R | T | E | V | A | E | L | E | Q | V | R | L | E | N | E | V | S | Q | Y | E   | T | R | Y |
| Heptad repeat              |   |      |   |   |   |   |   |   |   |   |   |   | a | b | c | d  | e  | f                        | g | a | b | c | d | e | f | g | a | b | c | d | e | f | g | a | b | c | d | e | f | g | a | b | c | d | e | f   | g | a |   |

**c****d****e****Supplementary Figure 1. The LIC1 helix binds on both side of the CC1-box coiled coil.**

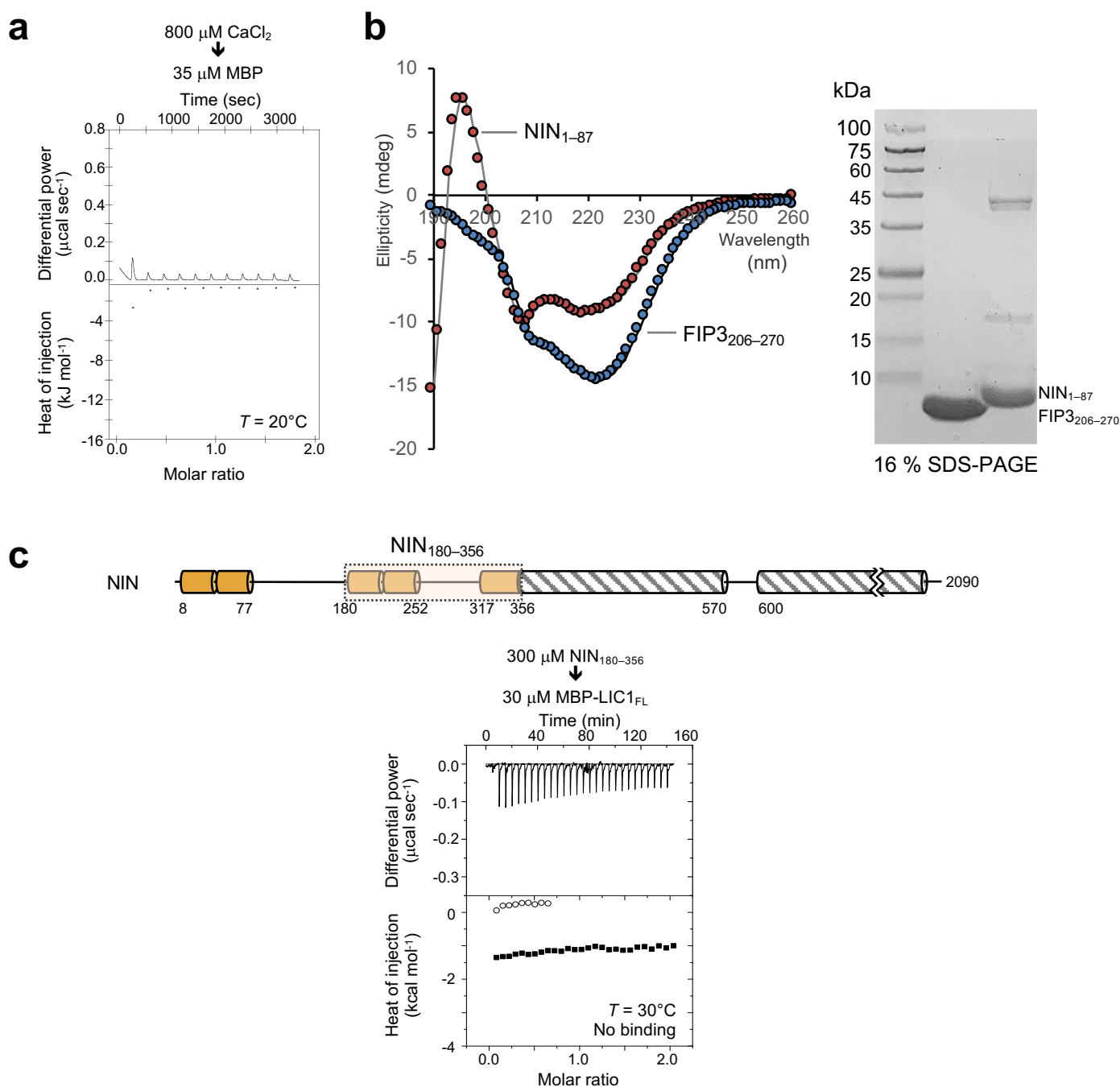
(a) Sequence alignment of the CC1-box region of several human adaptors (UniProt accession codes are listed with the name of each sequence). The graph below the alignment shows the per-residue conservation scores from analysis of 100 sequences of CC1-box-containing adaptors. Amino acids with conservation scores  $\geq 50\%$  are highlighted bold.

(b) Design of BICD2 WT/Y46D heterodimers stabilized via the C-terminal addition of the ZIP+/ZIP-leucine zipper<sup>24</sup>. The sequence alignment illustrates how electrostatic interactions between positively- and negatively-charged amino acids at alternating heptad positions e and g of the two coiled coil (orange striped boxes) stabilize the ZIP+/ZIP-heterodimer.

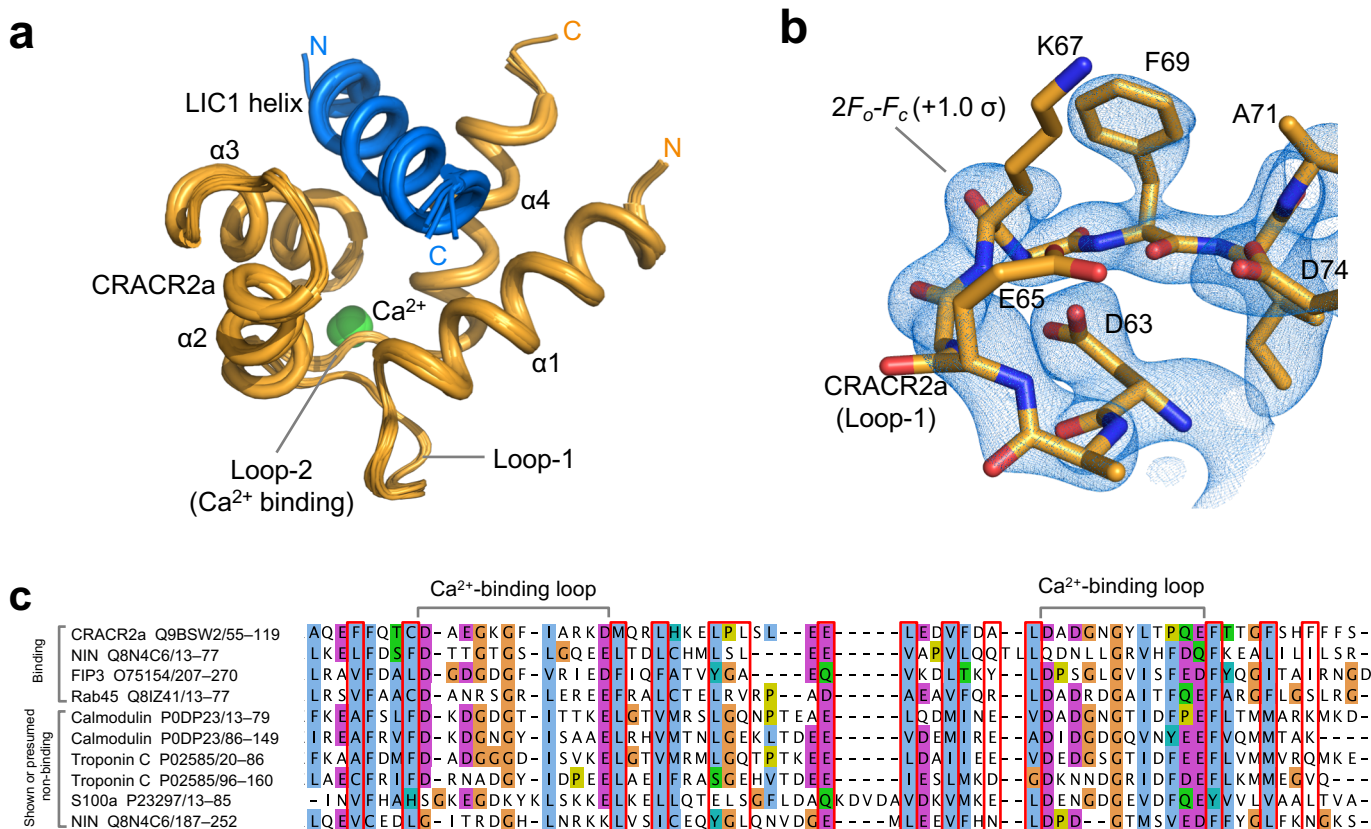
(c) SDS-PAGE analysis of the purified BICD2 WT<sub>ZIP-</sub>/Y46D<sub>ZIP+</sub> heterodimer (representative gel from n=2 independent experiments showing similar results). The two chains of the heterodimer have the same mass but migrate differently due to their different overall charges.

(d) SEC-MALS analysis of the purified BICD2 WT<sub>ZIP-</sub>/Y46D<sub>ZIP+</sub> heterodimer. The molar mass determined from light scattering ( $31.2 \pm 0.2$  kDa, right y-axis) and the UV absorption at 280 nm (left y-axis) are plotted as a function of the elution volume.

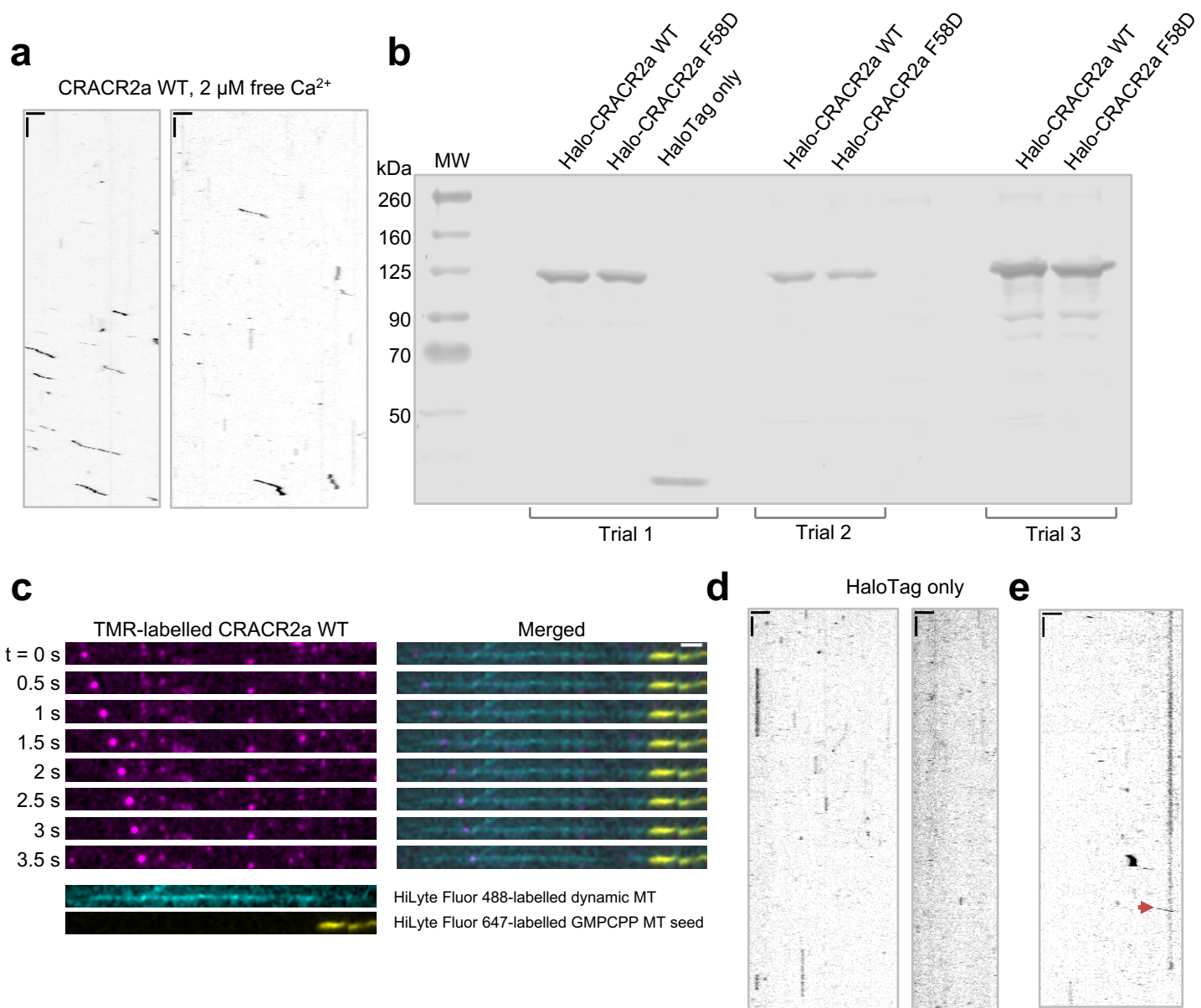
(e) Fitting of the ITC titration of LIC1<sub>433-458</sub> into BICD2 WT<sub>ZIP-</sub>/WT<sub>ZIP+</sub> using a one-site binding model. As indicated by the poor fit and high  $\chi^2$  value, the titration fits better to a two-site binding model with  $\chi^2$  values of 0.74 (Fig. 1f, left) compared to  $\chi^2=7.3$  for the one-site binding model.



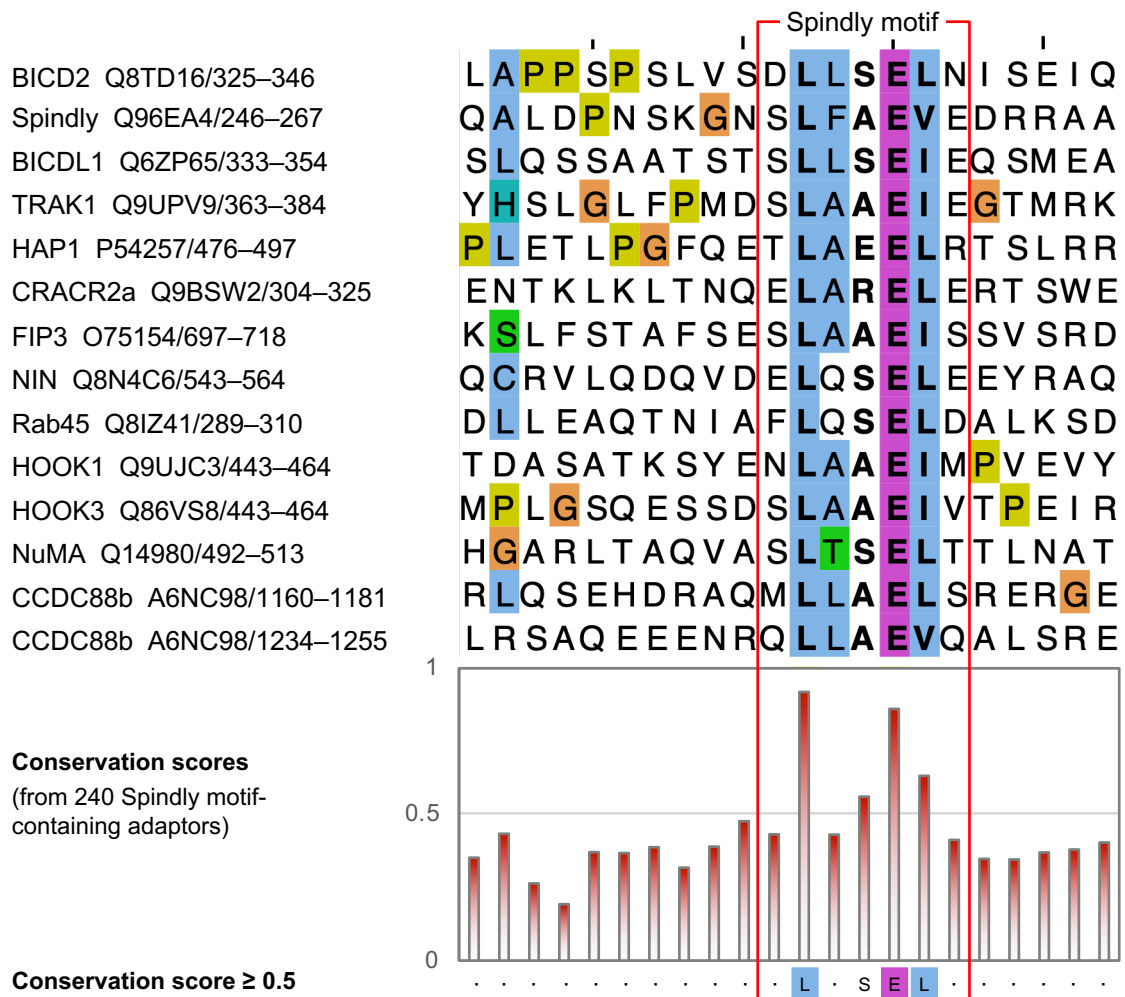
**Supplementary Figure 2. Additional experiments to characterize the interaction of LIC1 with EF-hand-containing adaptors.** (a) ITC titration of  $\text{CaCl}_2$  into maltose-binding protein (MBP). The experimental conditions are listed in the figure. The lack of change in the heat of injection indicates lack of binding and precludes fitting to a binding isotherm. (b) Circular dichroism (CD) spectra and SDS-PAGE analysis of constructs FIP3<sub>206-270</sub> and NIN<sub>1-87</sub> (representative gel from  $n=2$  independent experiments showing similar results). The CD spectra of both proteins are characteristic of  $\alpha$ -helical structures, with minima around 222 and 208 nm and no evidence of unfolding. (c) ITC titration of NIN<sub>180-356</sub> (containing three EF-hands, as shown in the diagram above) into MBP-LIC1<sub>FL</sub>. The experimental conditions are listed in the figure. Open symbols correspond to a control titration into buffer. The lack of change in the heat of injection indicates lack of binding and precludes fitting to a binding isotherm.



**Supplementary Figure 3. Interaction of CRACR2a with LIC1.** (a) Superimposition of the structures of the eight complex of CRACR2a<sub>47–122</sub> (orange) with LIC1<sub>433–458</sub> (blue) present in the asymmetric unit of the crystals (RMSDs < 0.4 Å for equivalent C $\alpha$  atoms among the eight complexes). (b) Close-up view of the first EF-hand loop (Loop-1 in part a) of CRACR2a that does not bind Ca<sup>2+</sup>. The 2Fo-Fc electron density map contoured at 1.0  $\sigma$  is shown as a blue mesh. (c) Comparison of the sequences of EF-hand pairs that either bind (top) or are presumed not to bind (bottom) the LIC1 helix. Note that only of NIN<sub>180–356</sub> (containing three EF-hand domains) has been experimentally shown not to bind the LIC1 helix (**Supplementary Fig. 2c**). Amino acids boxed red are found in the vicinity of the LIC1 helix in the structure of its complex with CRACR2a. We cannot identify any pattern of conservation among these residues that would allow us to predict whether an EF-hand pair can bind the LIC1 helix. The name of each sequence includes the UniProt accession code and the range of amino acids shown in the alignment.

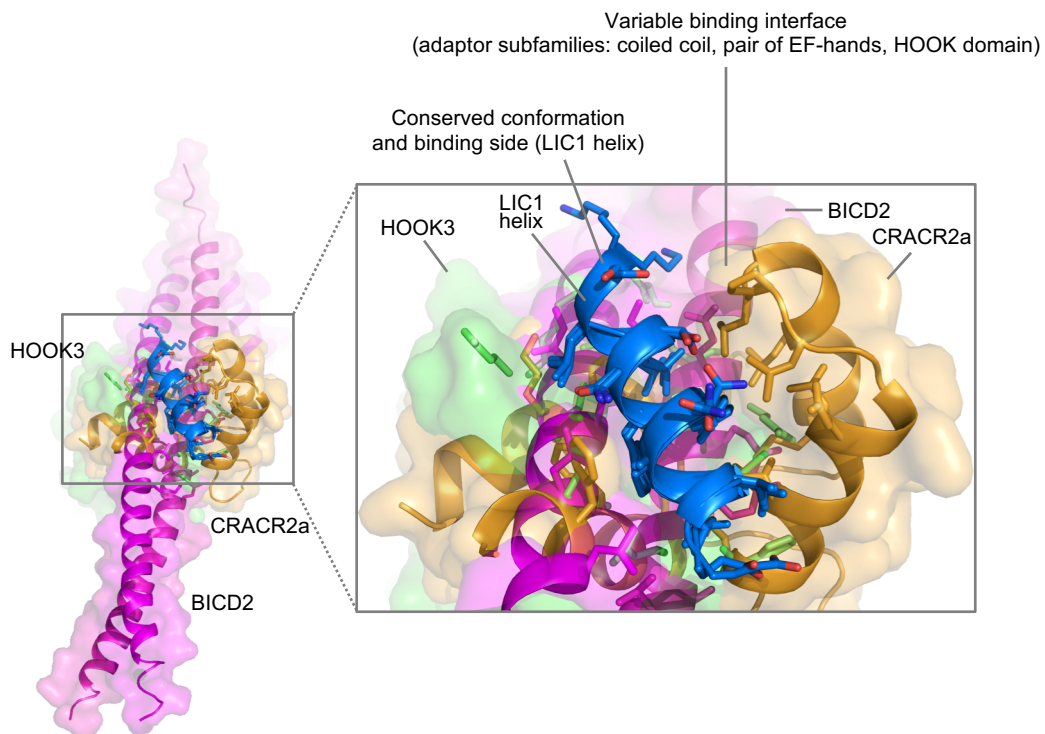


**Supplementary Figure 4. CRACR2a-containing complexes move toward the microtubule minus end.** (a) Additional representative kymographs (see also Fig. 5b in the main text) of the dynein-dynactin driven motility of single Halo-CRACR2a-positive particles (WT) analyzed by TIRF microscopy in the presence of 2  $\mu$ M free  $\text{Ca}^{2+}$ . (b) Western blot analysis of lysates of cells expressing either WT or mutant F58D TMR-labeled Halo-CRACR2a and from n=3 biological replicates used in single molecule motility assays. The blot was probed with polyclonal anti-Halo antibody (Promega, Madison, WI) at 1:1000 dilution and analyzed using an Odyssey CLx fluorescence imaging system (LI-COR Biosciences, Lincoln, NE). Source data provided in the Source Data file. (c) Time-lapse images of a TMR-labeled WT CRACR2a particle (magenta) moving along a HiLyte Fluor 488-labeled dynamic microtubule (MT; cyan) toward the HiLyte Fluor 647-labelled GMPCPP MT seed (yellow) at the minus end. The bright CRACR2a particle can be seen moving towards the MT minus end in both the merged (right) or TMR (561 nm, left) channels. Scale bar, 3  $\mu$ m. (d) Kymographs from a chamber containing TMR-labelled HaloTag alone, illustrating the lack of directed movement in the absence of CRACR2a (negative control). (e) Kymograph showing a single motile event (red arrow) with F58D mutant CRACR2a in the presence of  $\text{Ca}^{2+}$ . Scale bars: horizontal, 3  $\mu$ m; vertical, 5 sec.

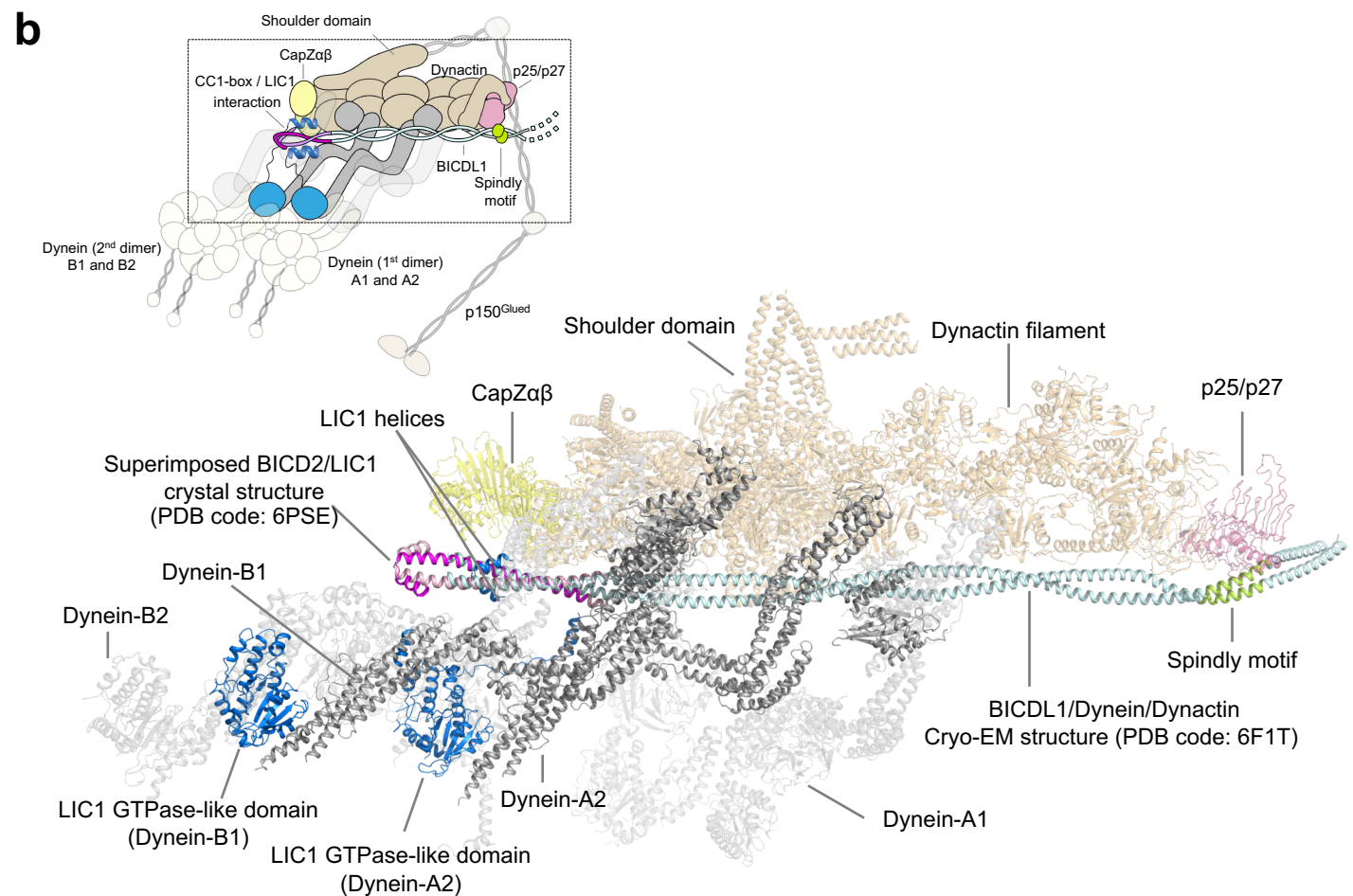
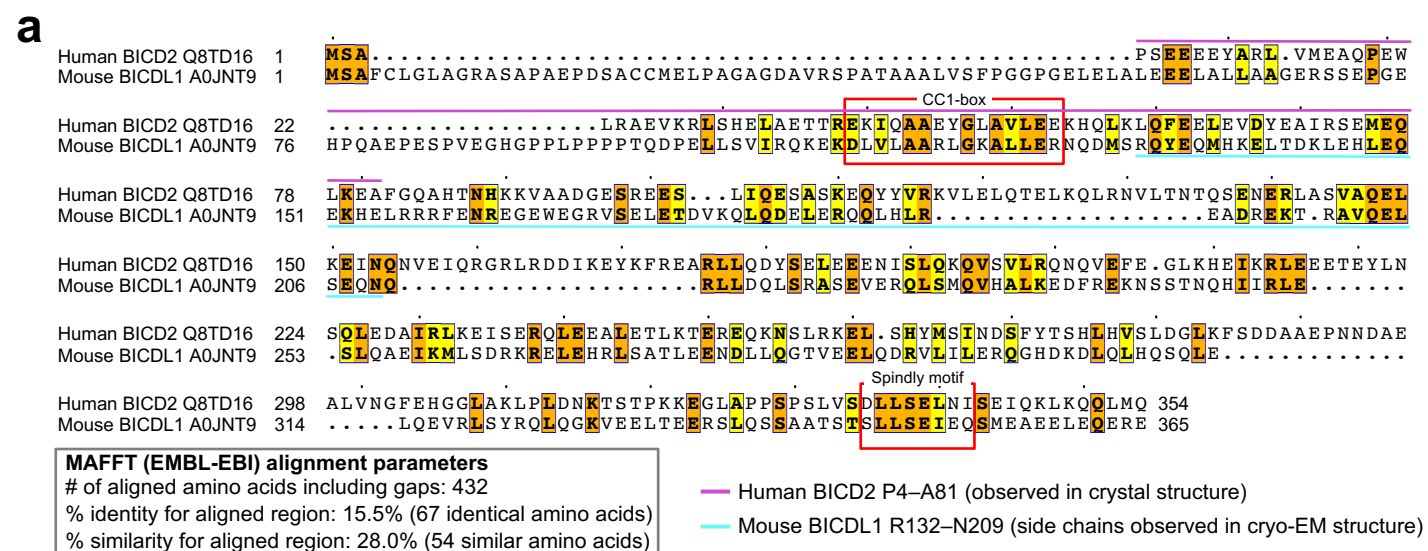


**Supplementary Figure 5. The Spindly motif.** Sequence alignment of the Spindly motif of several human adaptors. The UniProt accession codes and the range of amino acids shown in the alignment are listed with the name of each sequence. The graph below the alignment shows the per-residue conservation scores from analysis of 240 sequences of Spindly motif-containing adaptors. Amino acids with conservation scores  $\geq 50\%$  are highlighted (bold).





**Supplementary Figure 6. The LIC1-adaptor interface is conserved on the LIC1 side but variable on the adaptor side.** Superimposition of the three structures of adaptor-LIC1 complexes, including overall view (left) and close-up view (right) of the LIC1 helix (blue) binding interface. HOOK3, BICD2 and CRACR2a are colored green, magenta and orange, respectively. While the conformation and orientation of side chains is remarkably well-conserved for the LIC1 helix, there is no specific conservation of the binding interface among different adaptors subfamilies, other than the overall hydrophobic character of the interface.



**Supplementary Figure 7. Interaction of the LIC1 helix with CC1-box-containing adaptors within the adaptor-dynein-dynactin complex.** (a) Sequence alignment of the dynactin-binding region of human BICD2 and mouse BICDL1. The sequence identity is low (16%), but identical residues (bold) are identified along the length of the alignment, including within the CC1-box and Spindly motif (red boxes). A magenta line indicates the BICD2 region observed in the crystal structure of BICD2<sub>1-98</sub>-LIC1<sub>433-458</sub>. A cyan line below the alignment indicates the BICDL1 region for which side chains were observed in the 3.5-Å cryo-EM structure of BICDL1-dynein-dynactin<sup>9</sup> (residues 132–209; PDB code: 6PSE). (b) Cartoon (top) and atomic model (bottom) representations of the crystal structure of BICD2<sub>1-98</sub>-LIC1<sub>433-458</sub> superimposed onto the cryo-EM structure of BICDL1-dynein-dynactin based on the alignment shown in part a. Relevant subunits and interactions are labeled. The cryo-EM structure contains two dynein dimers bound (A and B). Based on the positions of the four LIC1 chains, it appears likely that each dynein dimer contributes one LIC1 helix (blue) to the interaction with the CC1-box, possibly explaining how two dynein dimers can be simultaneously activated by a single adaptor coiled coil dimer.



**Supplementary Table 1.** Dynein-dynactin adaptors and their cargoes

| Subfamily                                                     | Adaptor              | Evidence of LIC1 binding                                    | Evidence of dynein-dynactin binding/activation                                                           | Confirmed or unknown activator | Cellular cargo                                                                                                      |
|---------------------------------------------------------------|----------------------|-------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|--------------------------------|---------------------------------------------------------------------------------------------------------------------|
| <b>CC1-box-containing adaptors</b>                            |                      |                                                             |                                                                                                          |                                |                                                                                                                     |
| BICD family                                                   | BICD1                | UNK                                                         | Co-IP <sup>1</sup>                                                                                       | UNK                            | Golgi-derived vesicles <sup>2</sup>                                                                                 |
|                                                               | BICD2                | Co-IP <sup>3,4,5</sup> ITC <sup>6</sup> , SPR <sup>5</sup>  | TIRF (Reconstituted) <sup>7,8,9,10</sup><br>Relocation assay <sup>11</sup> , TIRF (Lysate) <sup>12</sup> | Confirmed                      | Golgi-derived vesicles <sup>2,13</sup> ,<br>Nuclear pore complexes <sup>14</sup> ,<br>Viral particles <sup>15</sup> |
|                                                               | BICDL1 (BICDR1)      | UNK                                                         | TIRF (Reconstituted) <sup>10,16</sup> ,<br>Relocation assay <sup>11</sup>                                | Confirmed                      | Rab6 vesicles <sup>17</sup>                                                                                         |
|                                                               | BICDL2 (BICDR1)      | UNK                                                         | Homology to BICDL1                                                                                       | UNK                            | Rab13 vesicles <sup>17</sup>                                                                                        |
| TRAK family                                                   | Spindly              | Co-IP <sup>3,5</sup> , SPR <sup>5</sup>                     | TIRF (Co-IP) <sup>8</sup>                                                                                | Confirmed                      | Kinetochore <sup>3</sup>                                                                                            |
|                                                               | TRAK1                | Co-IP <sup>5</sup>                                          | Co-IP <sup>18</sup>                                                                                      | UNK                            | Mitochondria <sup>18</sup>                                                                                          |
|                                                               | TRAK2                | UNK                                                         | Co-IP <sup>18</sup>                                                                                      | UNK                            | Mitochondria <sup>18</sup>                                                                                          |
|                                                               | HAP1                 | UNK                                                         | Co-IP <sup>19,20,21</sup>                                                                                | UNK                            | Autophagosomes <sup>22</sup> , BDNF-<br>containing vesicles <sup>23</sup>                                           |
| <b>HOOK domain-containing adaptors</b>                        |                      |                                                             |                                                                                                          |                                |                                                                                                                     |
| HOOK family                                                   | HOOK1                | ITC <sup>6</sup>                                            | TIRF (Lysate) <sup>12</sup>                                                                              | Confirmed                      | Early endosomes <sup>24,25</sup> ,<br>Clathrin-independent<br>cargoes <sup>26</sup>                                 |
|                                                               | HOOK2                | Co-IP <sup>27</sup>                                         | Relocation assay <sup>27</sup>                                                                           | UNK                            | Early endosomes <sup>25</sup> ,<br>Spermatid intramanchette <sup>28</sup> ,<br>Centrosomal proteins <sup>29</sup>   |
|                                                               | HOOK3                | Co-IP <sup>5,30</sup> , ITC <sup>6</sup> , SPR <sup>5</sup> | TIRF (Reconstituted) <sup>8,9,16</sup><br>TIRF (Lysate) <sup>6,12</sup>                                  | Confirmed                      | Early endosomes <sup>25</sup>                                                                                       |
| CCDC family                                                   | CCDC88A (Girdin)     | UNK                                                         | Co-IP <sup>31</sup>                                                                                      | UNK                            | UNK                                                                                                                 |
|                                                               | CCDC88B (HKRP3)      | UNK                                                         | Co-IP <sup>32</sup>                                                                                      | UNK                            | Secretory lysosomes <sup>32</sup>                                                                                   |
|                                                               | CCDC88C (Daple)      | UNK                                                         | Co-IP <sup>31</sup>                                                                                      | UNK                            | Planar cell polarity proteins <sup>33</sup>                                                                         |
|                                                               | NuMA                 | Co-IP <sup>34</sup>                                         | Co-IP <sup>32</sup>                                                                                      | UNK                            | Spindle microtubules <sup>35</sup>                                                                                  |
| <b>EF-hand-containing adaptors</b>                            |                      |                                                             |                                                                                                          |                                |                                                                                                                     |
| NIN family                                                    | NIN (Ninein)         | Co-IP <sup>5</sup>                                          | TIRF (Reconstituted) <sup>31</sup>                                                                       | Confirmed                      | Centrosome <sup>36</sup>                                                                                            |
|                                                               | NINL (Ninein-like)   | UNK                                                         | TIRF (Reconstituted) <sup>9, 31</sup>                                                                    | Confirmed                      | Rab8- and MICAL3-containing<br>vesicles <sup>37</sup>                                                               |
|                                                               | CRACR2a              | UNK                                                         | TIRF (Reconstituted) <sup>38</sup>                                                                       | Confirmed                      | ORAI1-STIM1 complex <sup>39</sup>                                                                                   |
|                                                               | Rab45                | UNK                                                         | TIRF (Reconstituted) <sup>38</sup>                                                                       | Confirmed                      | UNK                                                                                                                 |
| FIP family (Class II)                                         | Rab11-FIP3 (FIP3)    | Co-IP <sup>4,5,40,41</sup>                                  | TIRF (Co-IP) <sup>8</sup>                                                                                | Confirmed                      | Recycling endosomes <sup>41</sup>                                                                                   |
|                                                               | Rab11-FIP4 (FIP4)    | UNK                                                         | UNK                                                                                                      | UNK                            | Recycling endosomes <sup>41</sup>                                                                                   |
| <b>Other dynein-dynactin adaptors (RH1 domain-containing)</b> |                      |                                                             |                                                                                                          |                                |                                                                                                                     |
| JIP family                                                    | JIP1                 | UNK                                                         | Co-IP <sup>42</sup>                                                                                      | UNK (Unlikely)                 | Autophagosomes <sup>43</sup><br>Components of JNK signaling<br>pathway <sup>44</sup>                                |
|                                                               | JIP2                 | UNK                                                         | UNK                                                                                                      | UNK (Unlikely)                 | Components of JNK signaling<br>pathway <sup>44</sup>                                                                |
|                                                               | JIP3 (Sunday Driver) | UNK                                                         | Co-IP <sup>45</sup>                                                                                      | UNK (Unlikely)                 | Components of JNK signaling<br>pathway <sup>44</sup>                                                                |
|                                                               | JIP4                 | UNK                                                         | Co-IP <sup>46</sup>                                                                                      | UNK (Unlikely)                 | Components of JNK signaling<br>pathway <sup>44</sup>                                                                |
|                                                               | RILP                 | Co-IP <sup>4,47</sup>                                       | Co-IP <sup>48</sup>                                                                                      | UNK (Unlikely)                 | Autophagosomes <sup>49</sup><br>Lysosomes <sup>48</sup>                                                             |

**Abbreviations:** UNK, Unknown; Co-IP, Co-immunoprecipitation; ITC, Isothermal Titration Calorimetry; SPR, Surface Plasmon Resonance; BDNF, Brain-Derived Neurotrophic Factor; TIRF, Total Internal Reflection Fluorescence microscopy; MICAL3, [F-actin]-monooxygenase MICAL3; ORAI1, Ca<sup>2+</sup>-Release-Activated Ca<sup>2+</sup> Channel Protein-1; STIM1, Stromal Interaction Molecule-1; JNK, c-Jun N-terminal Kinase

**Supplementary Table 2. Primers used in this study**

| Construct                                  | Forward primer                                             | Reverse primer                                                           |
|--------------------------------------------|------------------------------------------------------------|--------------------------------------------------------------------------|
| BICD2 <sub>1-98</sub>                      | 5' cgcgatccgaaaacctgtatttcaggga<br>atgccgcgcgcgcgga        | 5' ctctcgacttagctctcaccatcagcagcca                                       |
| CRACR2a <sub>47-122</sub>                  | 5' cgcgatccgaaaacctgtatttcag<br>ggaggccagctagctatgctg      | 5' gcgtcgacttaattctggctgaagaagaagtac                                     |
| FIP3 <sub>206-270</sub>                    | 5' ggaattcgagaacctgtatttcaaagccgc<br>ctccgagccgtgttc       | 5' ctctcgacttaatctccgtttctgatggctgtg                                     |
| NIN <sub>180-356</sub>                     | 5'cgcgatccgaaaacctgtatttcagggacaagac<br>tgatagaagagaaactgc | 5' gcgtcgacgctgttcttgtaacaaaagtcat                                       |
| NIN <sub>1-87</sub>                        | 5' cgcgatccgaaaacctgtatttcaggga atggatgag<br>gtggagcagg    | 5' gcgtcgacttctgaaagtgttctcattgac                                        |
| BICD2 <sub>1-98</sub> Y46D                 | 5' : ccgccagtcctcctcgccgcctg                               | 5' : caggcgccgaggtgactggcgg                                              |
| BICD2 <sub>1-98</sub> D46Y                 | 5' : caggcgccgagtgactggcgg                                 | 5' : ccgccagtcatactcgccgcctg                                             |
| BICD2 <sub>1-98</sub> WT <sub>ZIP-</sub>   | 5' cgggatccgaaaacctgtatttcag                               | Rev1: 5' gcggtgttcgctggcgcagaaacgcgcgcgaatttcaggct<br>ctcaccatcagcagcc   |
|                                            |                                                            | Rev2: 5' caggcgctgcacttctgttccagttccgacctcggtgcgcagc<br>gcggtgttcgctg    |
|                                            |                                                            | Rev3: 5' gcgtcgacttaatagcgggttcatactggctcactcgtttc<br>caggcgctgcacttc    |
| BICD2 <sub>1-98</sub> Y46D <sub>ZIP+</sub> | 5' ggattccatatggaaaacctgtatttcag                           | Rev1: 5' gcggtgttttcgcttccagaaacgcgcgtcaatttcag<br>gctctcaccatcagcagcc   |
|                                            |                                                            | Rev2: 5' gcgctgcacgcgtggcgcagttccgccacgcgggttccagc<br>gcggtgttttcgcttc   |
|                                            |                                                            | Rev3: 5' gcgtcgacttaatagcgggtgcgatactggctcacgcgggtgcgc<br>aggcgctgcacgcg |
| LIC1 <sub>FL</sub>                         | 5' cgcgatccgaaaatctgtatttccaatc<br>catggcgccgtgggg         | 5' acggtcgactcaagaagcttctctccgtaggagat                                   |
| LIC1 <sub>1-461</sub>                      | 5' cgcgatccgaaaatctgtatttccaatc<br>catggcgccgtgggg         | 5' ctctcgacgcctctggagagccagctctttta                                      |
| CRACR2a <sub>FL</sub> (TIRF)               | 5' tagaattcagagctatggctgccctgacgggaggga                    | 5' tatgagctcccaggaagtccgctccag                                           |
| CRACR2a <sub>FL</sub> F58D<br>(TIRF)       | 5' gaaggcacaggaggacttccagacctgtg                           | 5' cacaggtctgaaagtctctgtgccttc                                           |

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