Supplemental Materials

Molecular Biology of the Cell

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Supplemental materials (3 movie files and 8 figures)

Movie S1. JIP1-dependent efficient axonal transport of APP cargo.

Movie **S1A** shows EGFP-APP cargo transport in a primary cultured neuron from a wild-type mouse (related to Fig. 1A). Movie **S1B** shows EGFP-APP cargo transport in a primary cultured neuron from a JIP1-KO mouse (related to Fig. 1B). Movie **S1C** shows EGFP-APP cargo transport in a primary cultured neuron from a JIP1-KO mouse with excess expression of JIP1b (related to Fig. 1C).

Movie S2. Axonal transport of APP cargo in JIP1-/- mouse and APP T668A knock-in mouse neurons.

Movie **S2A** shows EGFP-APP cargo transport in a primary cultured neuron from a JIP1-KO mouse with excess expression of JIP1bΔPI (related to Fig. 2A). Movie **S2B** shows EGFP-APP cargo transport in a primary cultured neuron from a JIP1-KO mouse with excess expression of JIP1b R156G/P157G (related to Fig. 2B). Movie **S2C** shows EGFP-APP T668A cargo transport in a primary cultured neuron from a APP Thr668Ala-KI mouse (related to Fig. 2C).

Movie S3. Axonal transport of APP cargo in JIP1-/-neurons expressing JIP1b mutant.

Movie **S3A** shows EGFP-APP cargo transport in a primary cultured neuron from a JIP1-KO mouse with excess expression of JIP1b Δ351-514 (related to Fig. 5A). Movie **S3B** shows EGFP-APP cargo transport in a primary cultured neuron from a JIP1-KO mouse with excess expression of JIP1b Δ370-402 (related to Fig. 5B). Movie **S3C** shows EGFP-APP cargo transport in a primary cultured neuron from a JIP1-KO mouse with excess expression of JIP1b Δ465-483 (related to Fig. 5C). Movie **S3D** shows EGFP-APP cargo transport in a primary cultured neuron from a JIP1-KO mouse with excess expression of JIP1b Y705A (related to Fig. 5D).

Figure S1. Interaction of JIP1b mutants with APP and JNK.

(A) Structure of the N-terminal HA-tagged JIP1b mutants used in this assay. Numbers represent amino acid positions. JBD, JNK-binding motif; SH3, Src homology domain

3; PI, phosphotyrosine interaction domain. Asterisks indicate positions of glycine substitution for arginine 156 and proline 157. **(B, C)** Interaction of APP with JIP1b. COS7 cells were transiently transfected with pcDNA3.1 plasmid encoding the indicated N-HA-tagged JIP1b mutant in the presence of pcDNA3.1-FLAG-APP695 **(B)** or pcDNA3.1-FLAG- JNK1α1 **(C)**. The "-" indicates empty vector alone. Co-immunoprecipitation with anti-FLAG antibody (IP) and immunoblotting (lysate) are described in Fig. 3. **(D)** Interaction of KLC1 C46 with JIP1b. Structures of C-terminal EGFP-tagged mouse KLC1 proteins and N-terminal FLAG-tagged mouse JIP1b are shown in Fig. 3A. Co-immunoprecipitation assay of KLC1-EGFP (FL), KLC1 C46 (C46) and EGFP alone (EGFP) with FLAG-JIP1b are shown. The immunoprecipitates (IP) and lysate samples were analyzed by immunoblotting with anti-EGFP and anti-FLAG antibodies. Numbers shown at the left of the panel indicate molecular weight standards (kDa). Numbers shown below the panel indicate lane numbers.

Figure S2. Interaction of JIP1b regions with KLC1 regions.

(A) Structures of N-terminal FLAG-tagged mouse JIP1b proteins with or without a C-terminal EGFP tag. Summary of JIP1b region interactions with KLC1 (FL), KLC1 TPR domains (TPR), and KLC1 N200 region (N200) is shown on the right, classified as binding (+) and non-binding (-) in a co-immunoprecipitation assay. N.D., not determined. (**B-D**) Co-immunoprecipitation assays of JIP1b regions with KLC1 (FL), TPR, and N200. The "-" indicates empty vector alone. The immunoprecipitate (IP) and lysate samples were analyzed by immunoblotting with anti-HA and anti-FLAG antibodies. Numbers shown at the left of the panel indicate molecular weight standards (kDa). Numbers shown below the panel indicate lane numbers.

Figure S3. Interaction of JIP1b proteins with or without C11 region to N200 of KLC1. (A) Structures of N-terminal FLAG-tagged mouse JIP1b proteins with or without a C-terminal 11 amino acids used in this assay. (**B**) Co-immunoprecipitation assays of JIP1b proteins with N200 of KLC1. The "-" indicates empty vector alone. The immunoprecipitate (IP) and lysate samples were analyzed by immunoblotting with anti-HA and anti-FLAG antibodies. Numbers shown at the left of the panel indicate molecular weight standards (kDa).

Figure S4. In vitro interaction of JIP1b$^{351-514}$ with N200 of KLC. GST-JIP1b$^{351-514}$, The purified GST-JIP1b$^{351-514}$, GST-JIP1bΔ351-514 and GST proteins (10 μg) were incubated with purified KLC1 (0.8 μg protein) or KLC1 N200 (1.6 μg) and associated proteins were recovered with glutathione beads and subjected to Western blot analysis with anti-KLC1 UT109 (upper) and anti-GST antibodies (lower), along with protein mixtures (Input).

Figure S5. Interaction of internal deletion JIP1b mutants with KLC1 proteins.
(A) Structures of N-terminal FLAG-tagged internal deletion mouse JIP1b proteins. Summary of JIP1b interaction with KLC1 (FL), KLC1 TPR domains (TPR), and the KLC1 N200 region (N200) is shown on the right, classified as binding (+) and non-binding (-) in a co-immunoprecipitation assay. (**B, C**) Co-immunoprecipitation of JIP1b mutants with KLC1 (FL), TPR, and N200. The "-" indicates empty vector alone. The immunoprecipitation (IP) and lysate samples were analyzed by immunoblotting with anti-HA and anti-FLAG antibodies. Numbers shown at the left of the panel indicate molecular weight standards (kDa). Numbers shown below the panel indicate lane numbers.

Figure S6. Interaction of JIP1b fragments with KLC1.
(**A**) Structures of the N-terminal FLAG-tagged mouse JIP1b fragments with C-terminal EGFP. Summary of JIP1b interactions with KLC1 (FL) and the KLC1 N200 region (N200) is shown on the right, classified as binding (+), weak binding (±), and non-binding (-) in a co-immunoprecipitation assay. (**B, C**) Co-immunoprecipitation of JIP1b fragments with KLC1 (FL) and N200. FLAG-EGFP indicates plasmids expressing tag alone, and the "-" indicates empty vector alone. The immunoprecipitation (IP) and lysate samples were analyzed by immunoblotting with anti-HA and anti-FLAG antibodies. Numbers shown at the left of the panel indicate molecular weight standards (kDa). Numbers shown below the panel indicate lane numbers.

Figure S7. Interaction of internal deletion JIP1b mutants with KLC1.
(A) Structures of the N-terminal FLAG-tagged internal deletion mouse JIP1b mutants. Asterisk indicates a position of alanine substitution for tyrosine (Y705A). Summary

of JIP1b interactions with KLC1 (FL), KLC1 TPR domains (TPR), and the KLC1 N200 region (N200) is shown on the right, classified as binding (+) and non-binding (-) in co-immunoprecipitation assay. (**B**) Co-immunoprecipitation of JIP1b proteins with KLC1 (FL), TPR, and N200. The "-" indicates empty vector alone. The immunoprecipitates (IP) and lysate were analyzed by immunoblotting with anti-HA and anti-FLAG antibodies. Numbers shown at the left of the panel indicate molecular weight standards (kDa). Numbers shown below the panel indicate lane numbers.

Fig. S8. Formation of the kinesin-1 complex composed of JIP1b, KLC, and KHC. COS7 cells were transiently expressed with FLAG-JIP1b WT or FLAG-JIP1bΔC11 (**A**), or HA-JIP1bWT or HA-JIP1bY705A (**B**) in the presence or absence of KHC and HA-KLC1 (**A**), or KHC-FLAG and Myc-KLC1 (**B**). +, indicated plasmid; -, mock plasmid. Cell lysates were immunoprecipitated with anti-FLAG antibody. The immunoprecipitates (IP) and lysate were analyzed by immunoblotting with anti-KHC, anti-FLAG, anti-HA and anti-KLC1 antibodies. Numbers shown at the left of the panel indicate molecular weight standards (kDa).

Figure S1

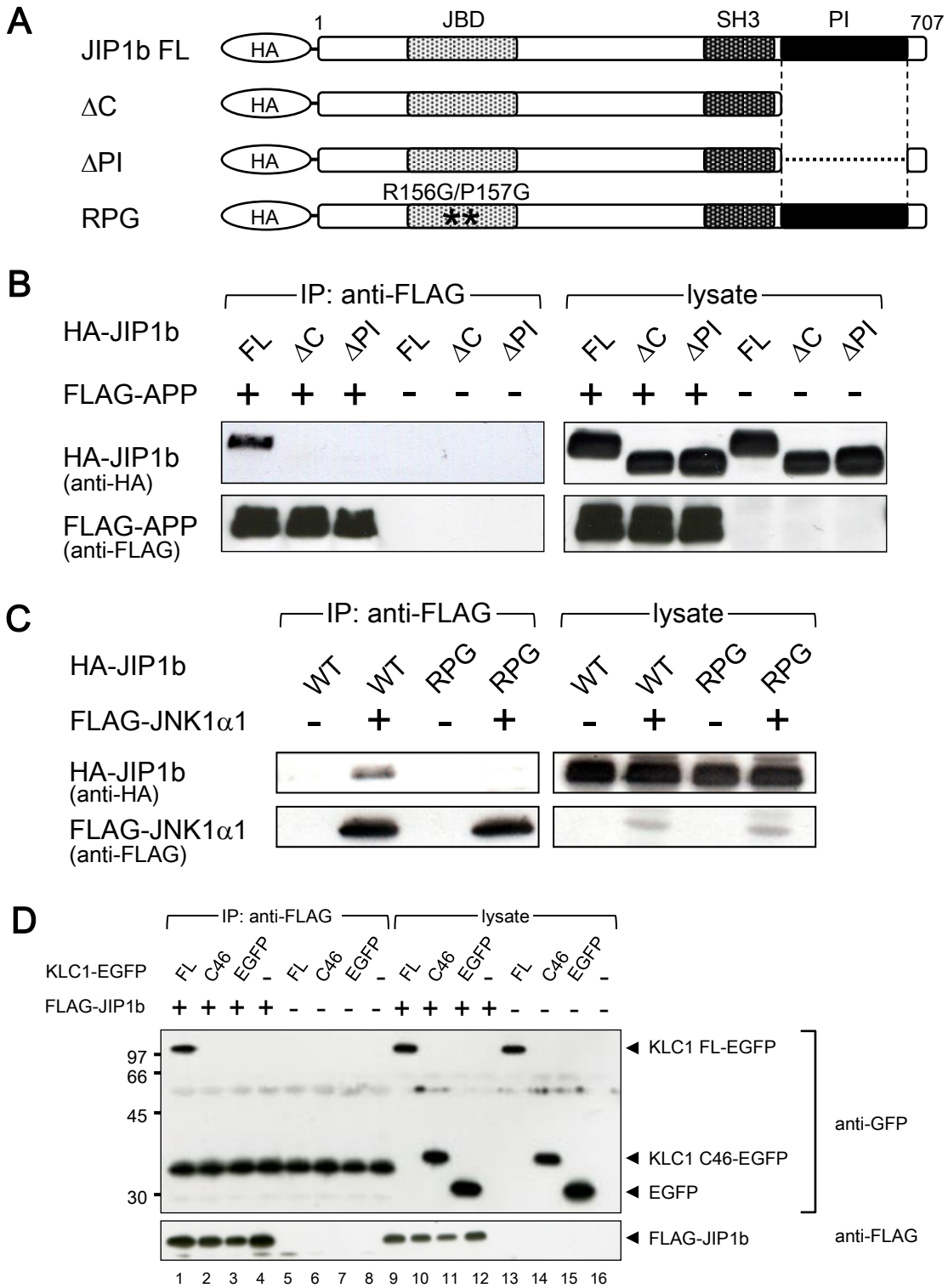

A
1
JBD
SH3
PI
707
JIP1b FL
HA
ΔC
ΔPI
R156G/P157G
RPG
B
IP: anti-FLAG
lysate
HA-JIP1b
FL
ΔC
ΔPI
FLAG-APP
HA-JIP1b
(anti-HA)
FLAG-APP
(anti-FLAG)
C
IP: anti-FLAG
lysate
HA-JIP1b
WT
RPG
FLAG-JNK1α1
HA-JIP1b
(anti-HA)
FLAG-JNK1α1
(anti-FLAG)
D
IP: anti-FLAG
lysate
KLC1-EGFP
FL
C46
EGFP
FLAG-JIP1b
97
66
45
30
KLC1 FL-EGFP
KLC1 C46-EGFP
EGFP
anti-GFP
FLAG-JIP1b
anti-FLAG
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Figure S2

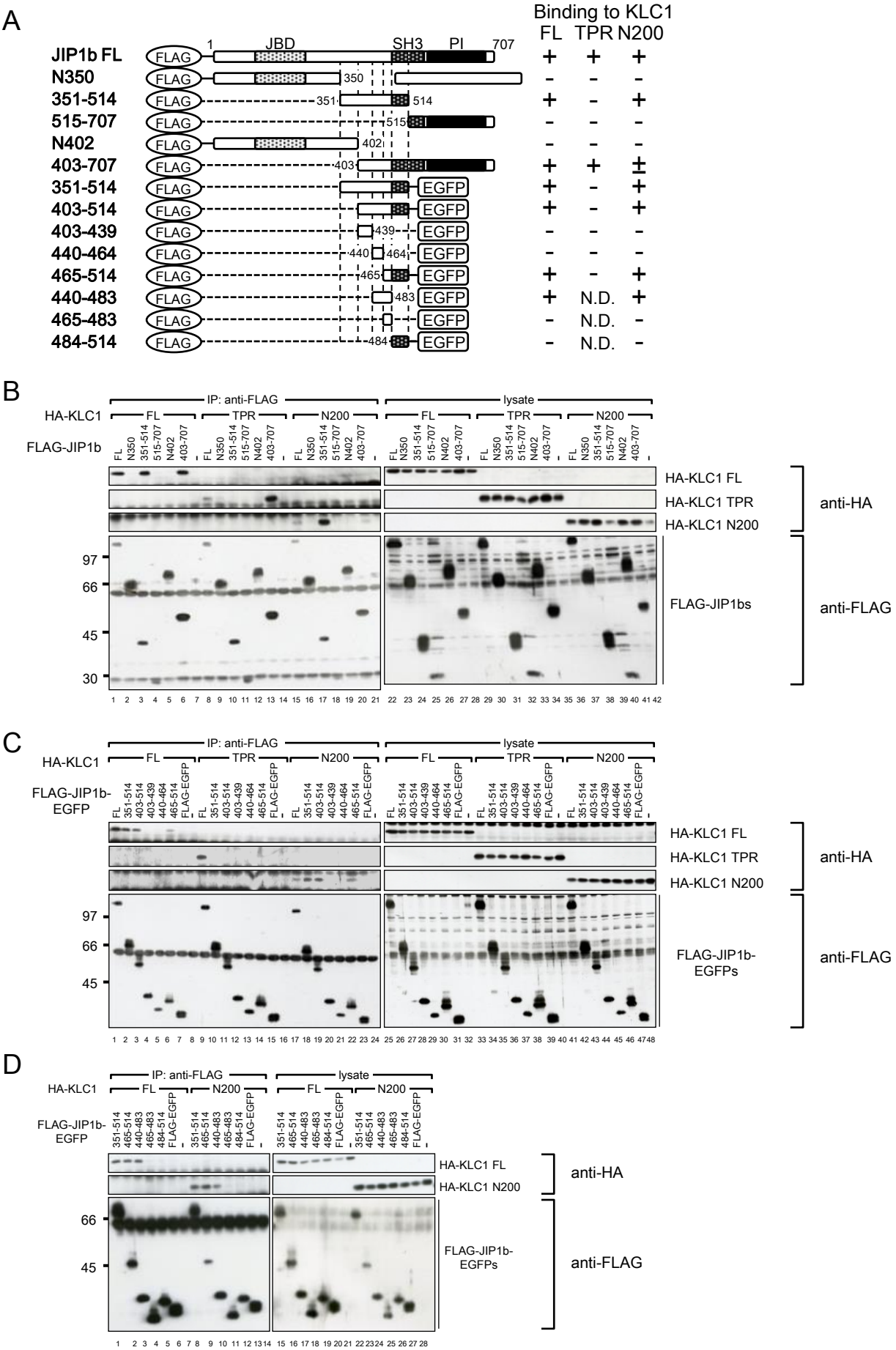

A
Binding to KLC1
FL TPR N200
JIP1b FL
N350
351-514
515-707
N402
403-707
351-514
403-514
403-439
440-464
465-514
440-483
465-483
484-514
FLAG
EGFP
JBD
SH3
PI
1
707
B
IP: anti-FLAG
lysate
HA-KLC1
FLAG-JIP1b
HA-KLC1 FL
HA-KLC1 TPR
HA-KLC1 N200
anti-HA
FLAG-JIP1bs
anti-FLAG
97
66
45
30
C
FLAG-JIP1b-EGFP
FLAG-JIP1b-EGFPs
D

Figure S3

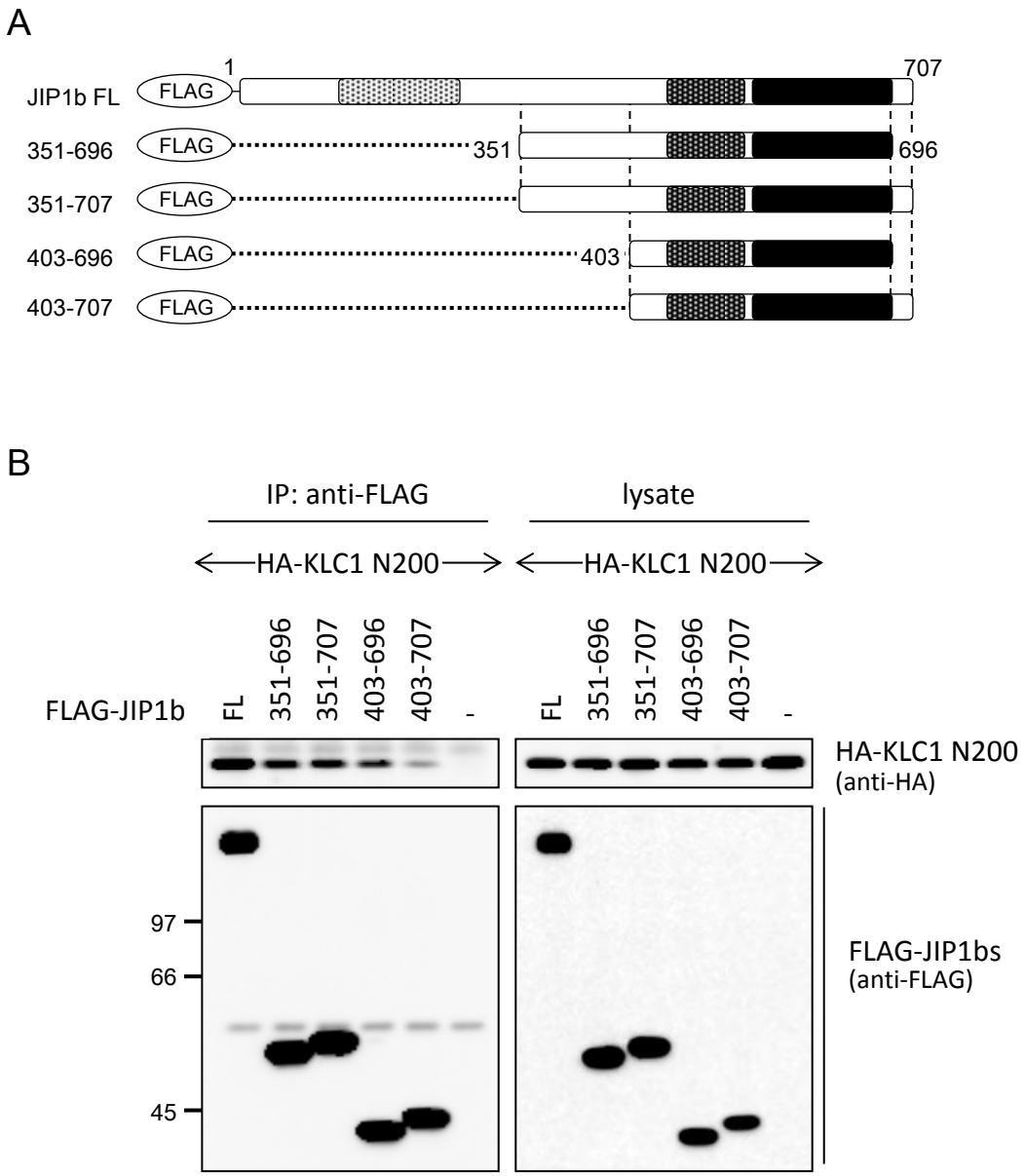

A
1
707
JIP1b FL
FLAG
351-696
351
696
351-707
403-696
403
403-707
B
IP: anti-FLAG
lysate
HA-KLC1 N200
HA-KLC1 N200
FLAG-JIP1b
FL
351-696
351-707
403-696
403-707
-
HA-KLC1 N200
(anti-HA)
97
66
45
FLAG-JIP1bs
(anti-FLAG)

Figure S4

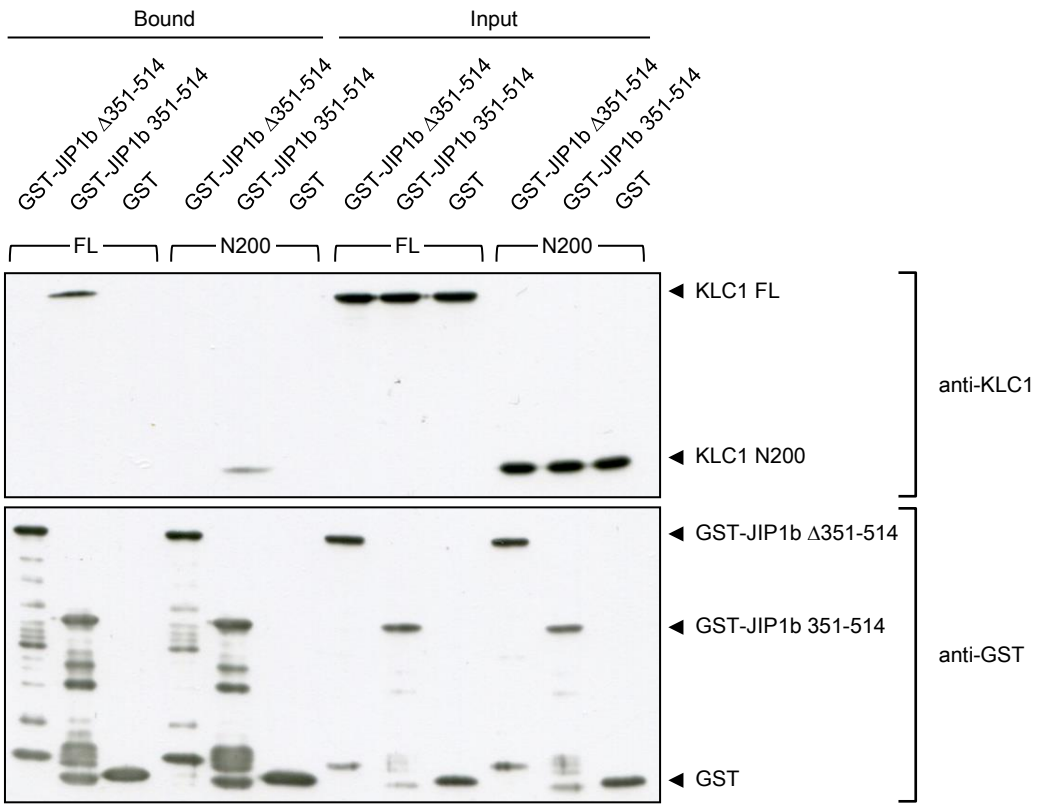

Bound
Input
GST-JIP1b Δ351-514
GST-JIP1b 351-514
GST
FL
N200
KLC1 FL
KLC1 N200
anti-KLC1
GST-JIP1b Δ351-514
GST-JIP1b 351-514
GST
anti-GST

Figure S5

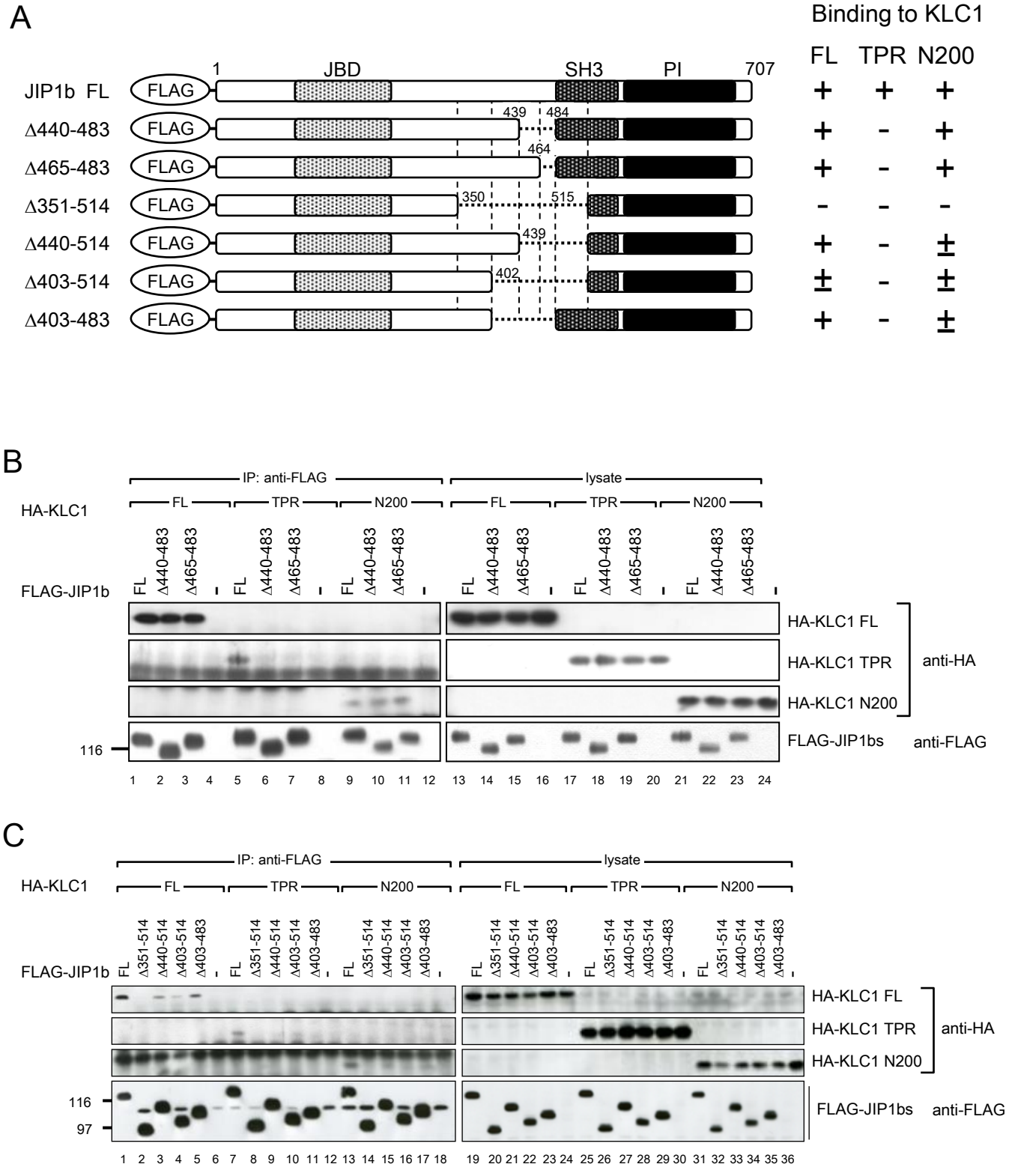

A
Binding to KLC1
FL TPR N200
1
JBD
SH3
PI
707
JIP1b FL
Δ440-483
Δ465-483
Δ351-514
Δ440-514
Δ403-514
Δ403-483
FLAG
439
484
464
350
515
402
B
IP: anti-FLAG
lysate
HA-KLC1
FL
TPR
N200
FLAG-JIP1b
HA-KLC1 FL
HA-KLC1 TPR
HA-KLC1 N200
anti-HA
FLAG-JIP1bs
anti-FLAG
116
C
IP: anti-FLAG
lysate
HA-KLC1
FL
TPR
N200
FLAG-JIP1b
HA-KLC1 FL
HA-KLC1 TPR
HA-KLC1 N200
anti-HA
FLAG-JIP1bs
anti-FLAG
116
97

Figure S6

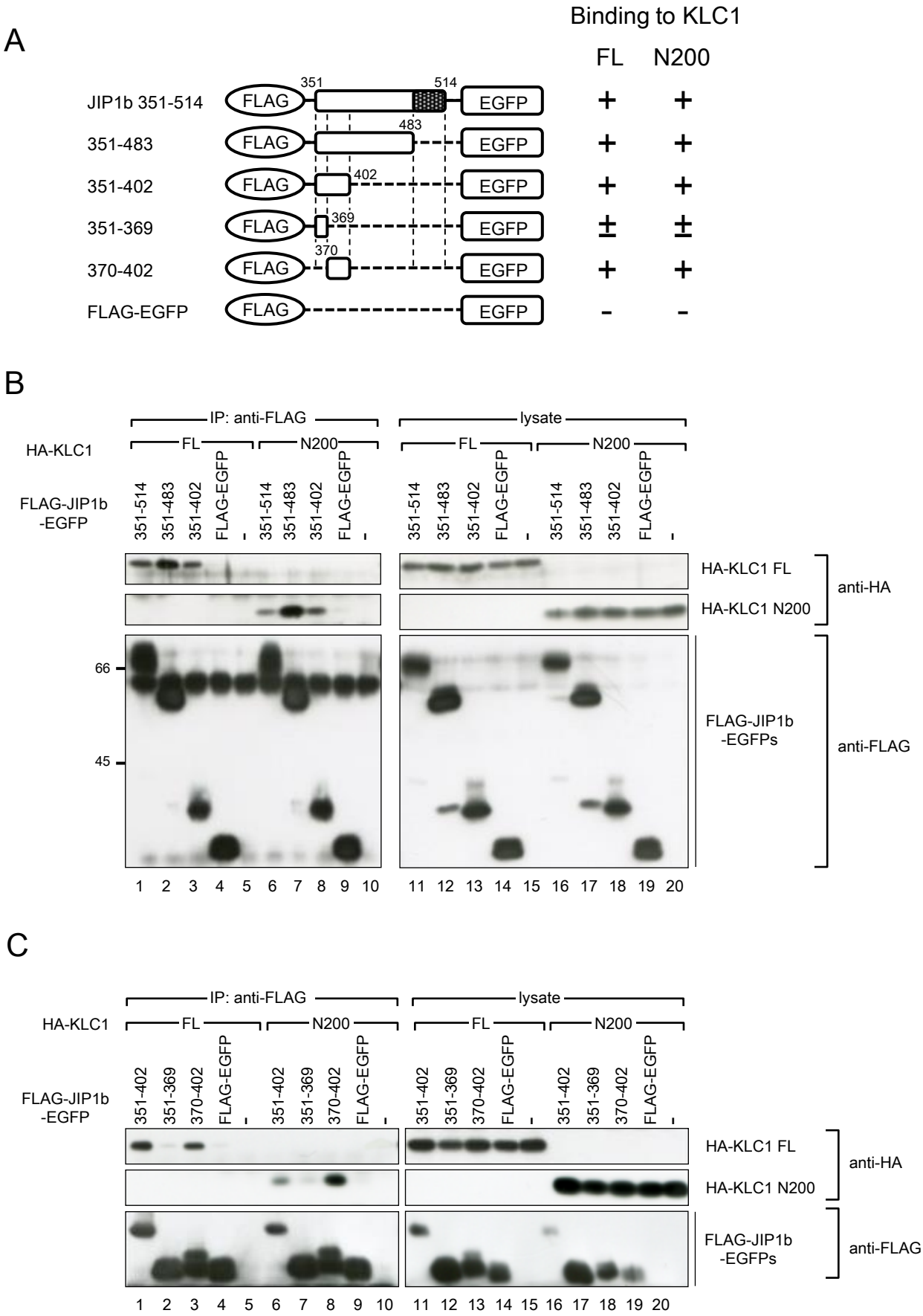
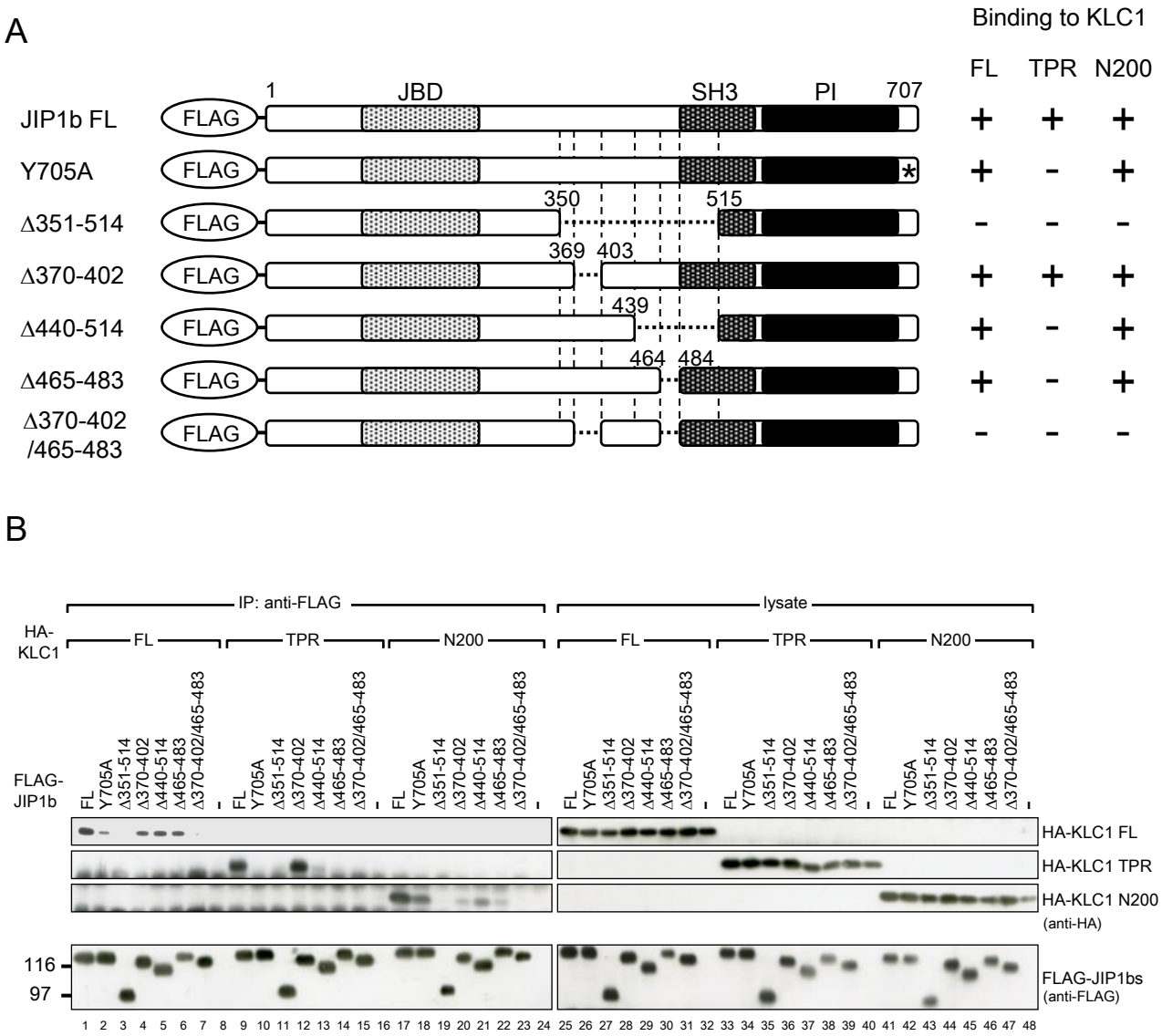

A
Binding to KLC1
FL
N200
JIP1b 351-514
351-483
351-402
351-369
370-402
FLAG-EGFP
FLAG
EGFP
351
514
483
402
369
370
+
±
-
B
IP: anti-FLAG
lysate
HA-KLC1
FL
N200
FLAG-JIP1b -EGFP
351-514
351-483
351-402
FLAG-EGFP
HA-KLC1 FL
HA-KLC1 N200
anti-HA
66
45
FLAG-JIP1b -EGFPs
anti-FLAG
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
C
IP: anti-FLAG
lysate
HA-KLC1
FL
N200
FLAG-JIP1b -EGFP
351-402
351-369
370-402
FLAG-EGFP
HA-KLC1 FL
HA-KLC1 N200
anti-HA
FLAG-JIP1b -EGFPs
anti-FLAG
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Figure S7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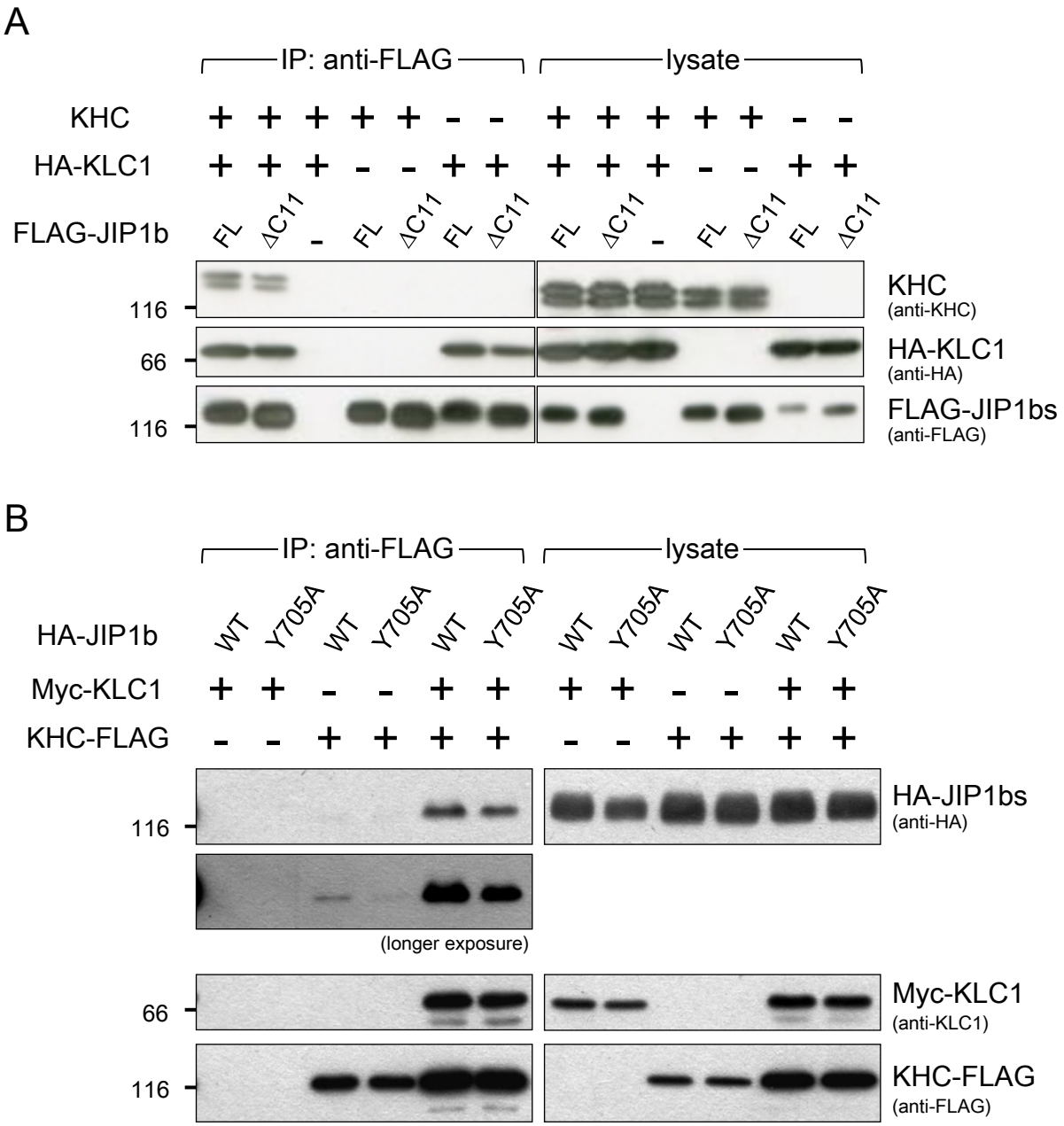
Binding to KLC1
FL TPR N200
1 JBD SH3 PI 707
JIP1b FL FLAG + + +
Y705A FLAG + - +
350 515
Δ351-514 FLAG - - -
369 403
Δ370-402 FLAG + + +
439
Δ440-514 FLAG + - +
464 484
Δ465-483 FLAG + - +
Δ370-402 /465-483 FLAG - - -
B
IP: anti-FLAG
lysate
HA-KLC1
FL
TPR
N200
FLAG-JIP1b
FL Y705A Δ351-514 Δ370-402 Δ440-514 Δ465-483 Δ370-402/465-483 -
HA-KLC1 FL
HA-KLC1 TPR
HA-KLC1 N200
(anti-HA)
116
97
FLAG-JIP1bs
(anti-FLAG)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48

Figure S8


A
IP: anti-FLAG
lysate
KHC
HA-KLC1
FLAG-JIP1b
FL
ΔC11
-
116
66
116
KHC
(anti-KHC)
HA-KLC1
(anti-HA)
FLAG-JIP1bs
(anti-FLAG)
B
IP: anti-FLAG
lysate
HA-JIP1b
WT
Y705A
Myc-KLC1
KHC-FLAG
116
(longer exposure)
66
116
HA-JIP1bs
(anti-HA)
Myc-KLC1
(anti-KLC1)
KHC-FLAG
(anti-FLAG)