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Title	Quantitative analysis of APP axonal transport in neurons: role of JIP1 in enhanced APP anterograde transport
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Supplemental Materials

Molecular Biology of the Cell

Chiba et al.

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 JIP1b Δ PI (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 Δ 351-514 (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 Δ 370-402 (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 Δ 465-483 (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- JNK1 α 1 **(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 JIP1b³⁵¹⁻⁵¹⁴ with N200 of KLC. GST-JIP1b³⁵¹⁻⁵¹⁴, The purified GST-JIP1b³⁵¹⁻⁵¹⁴, 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-JIP1b Δ C11 **(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

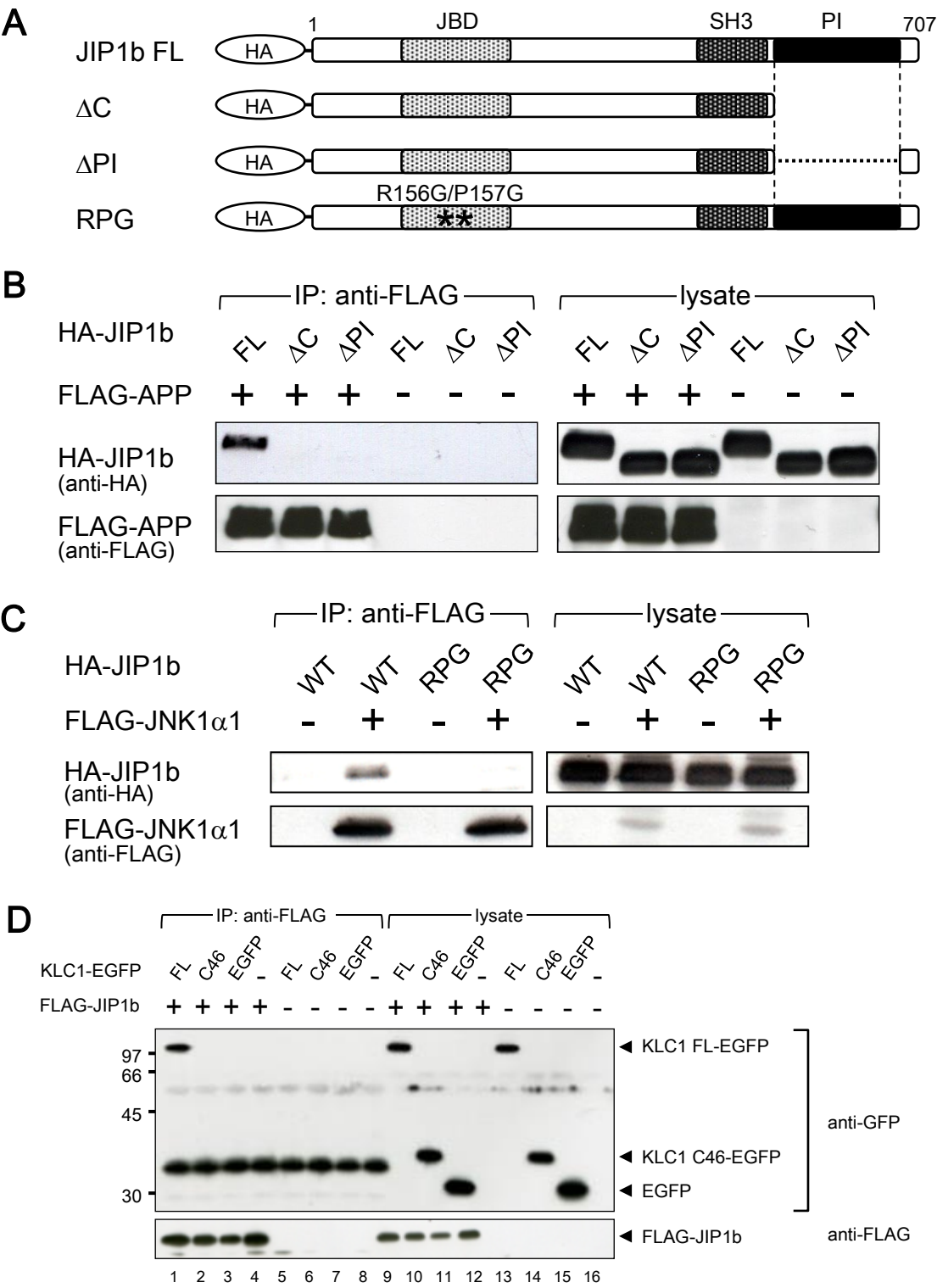


Figure S2

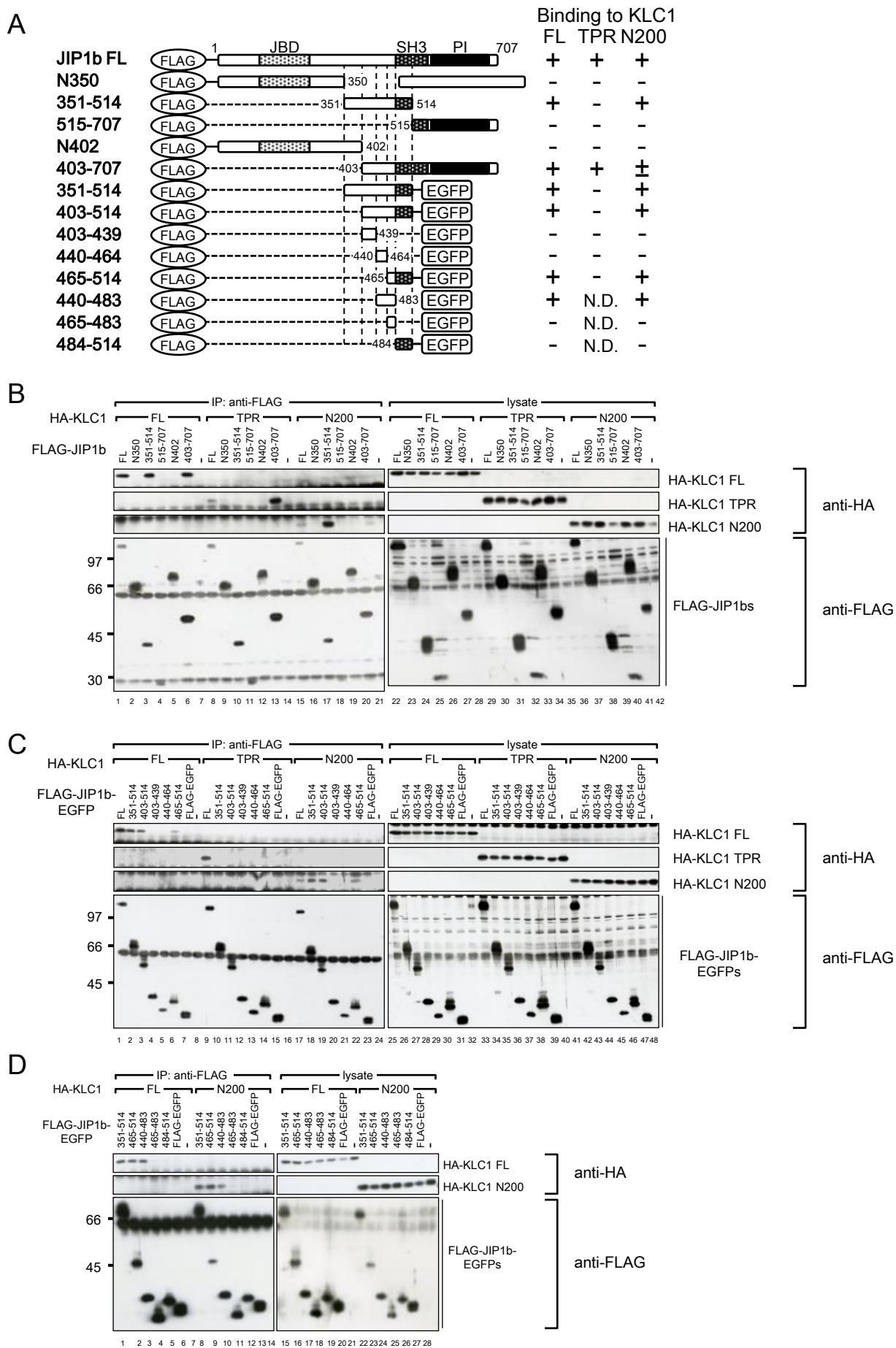
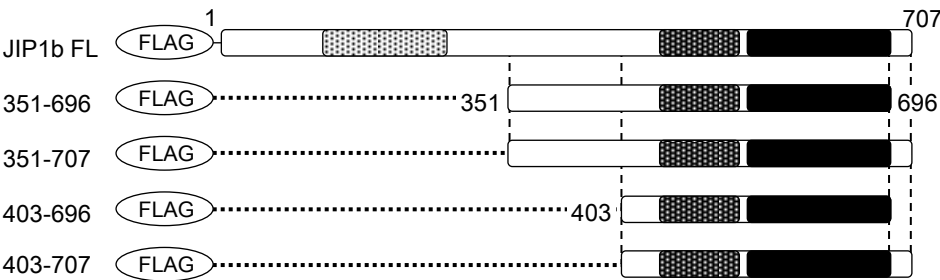


Figure S3

A



B

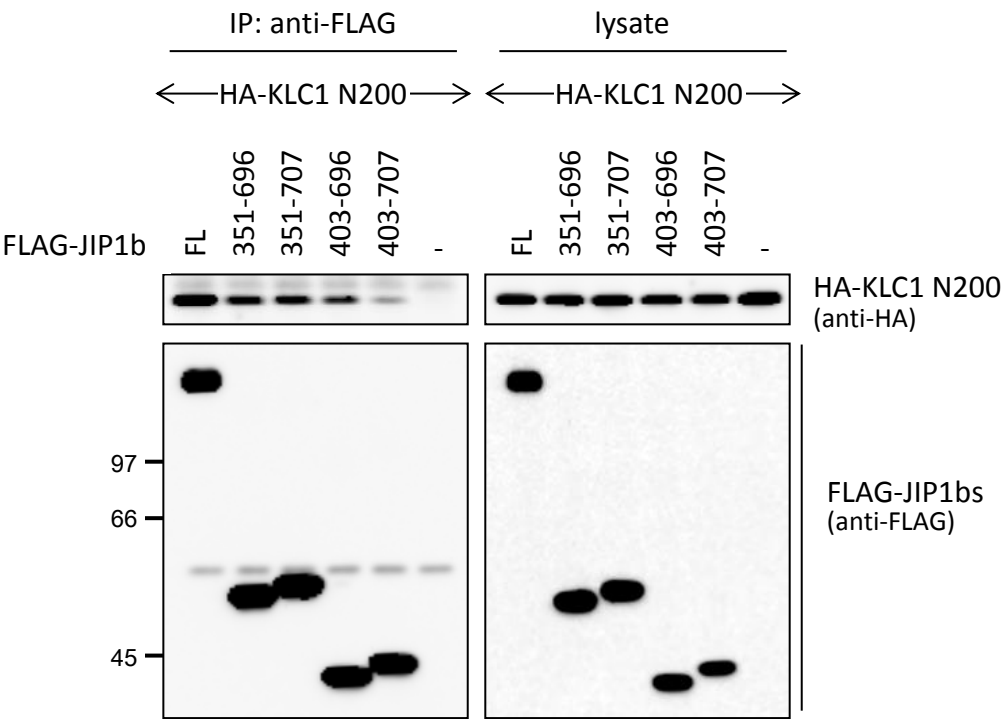


Figure S4

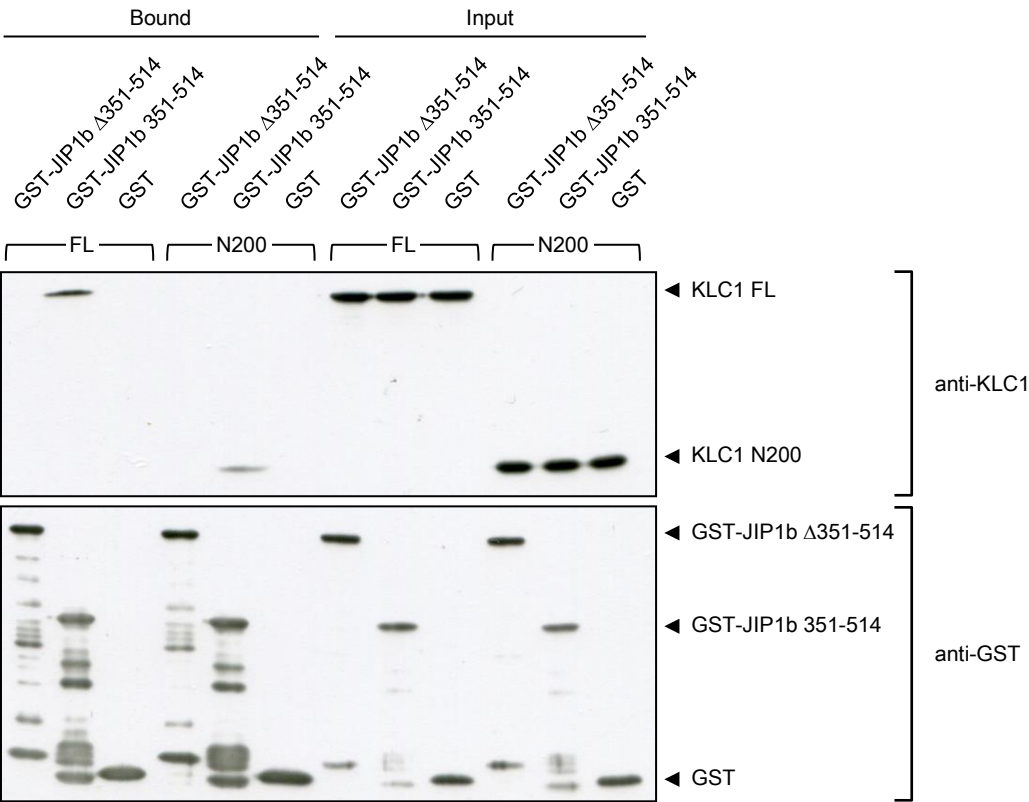


Figure S5

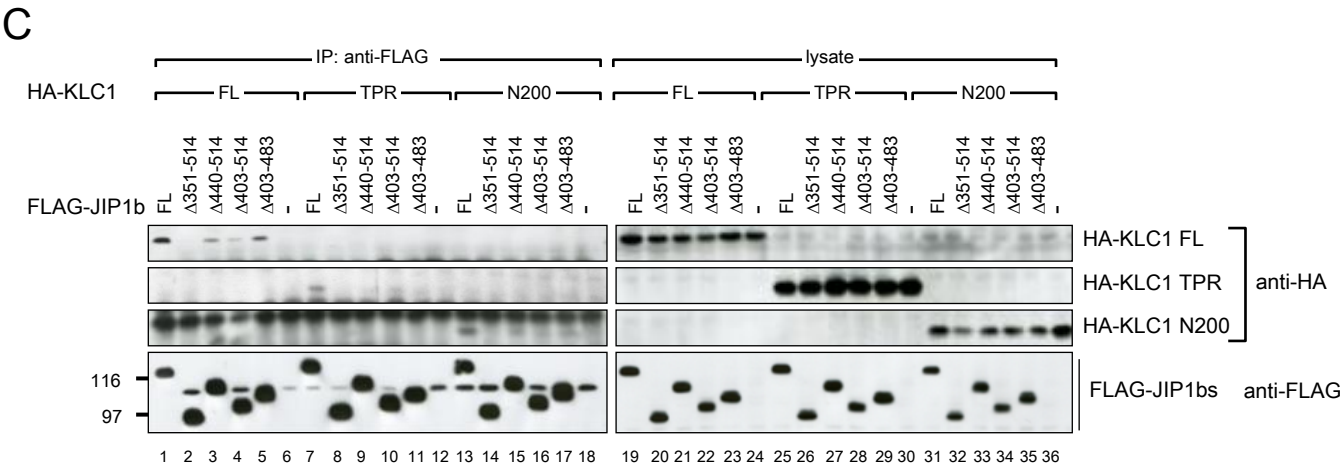
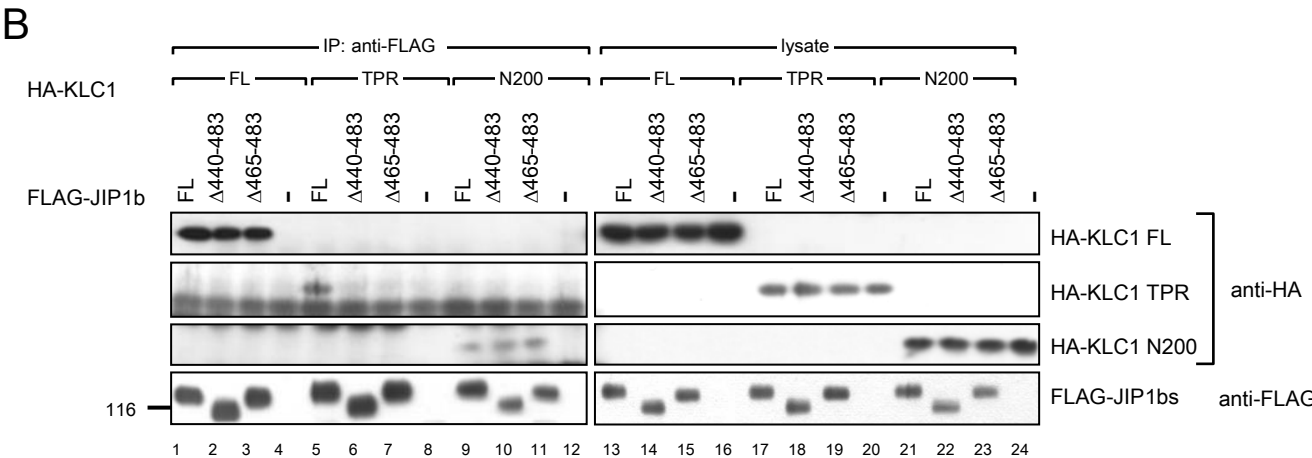
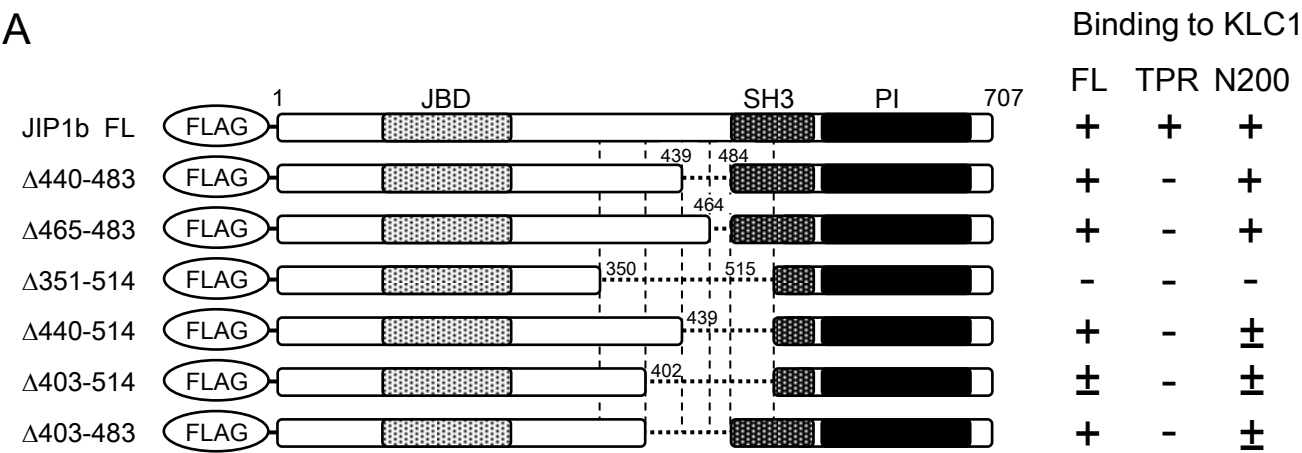


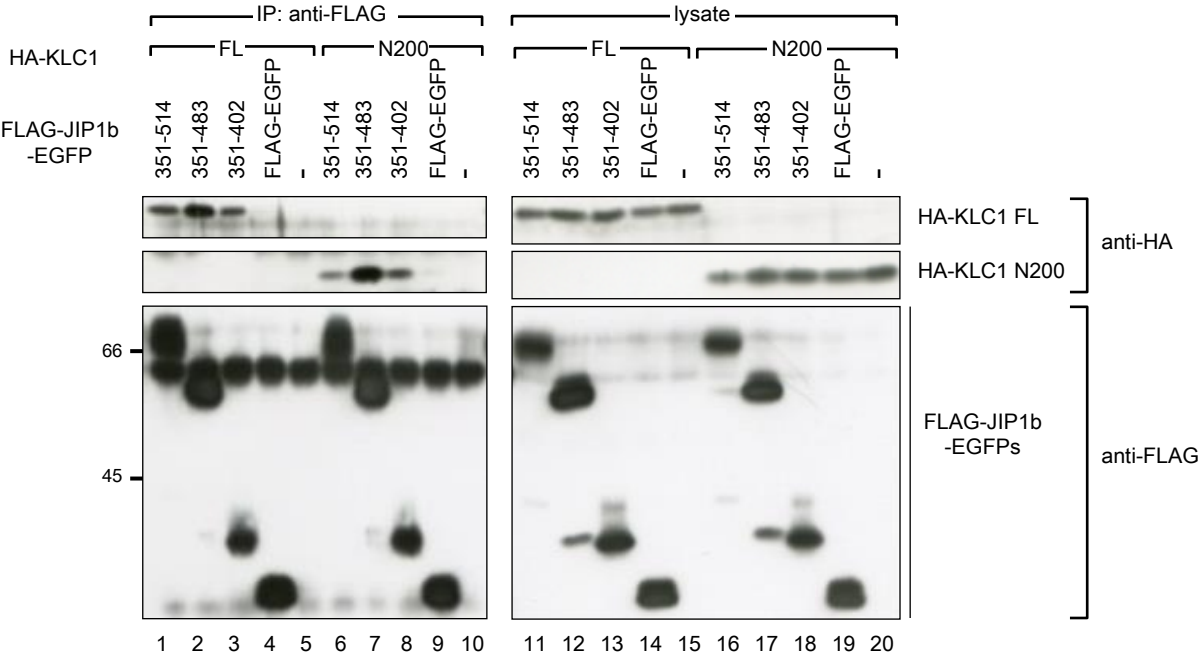
Figure S6

Binding to KLC1

A



B



C

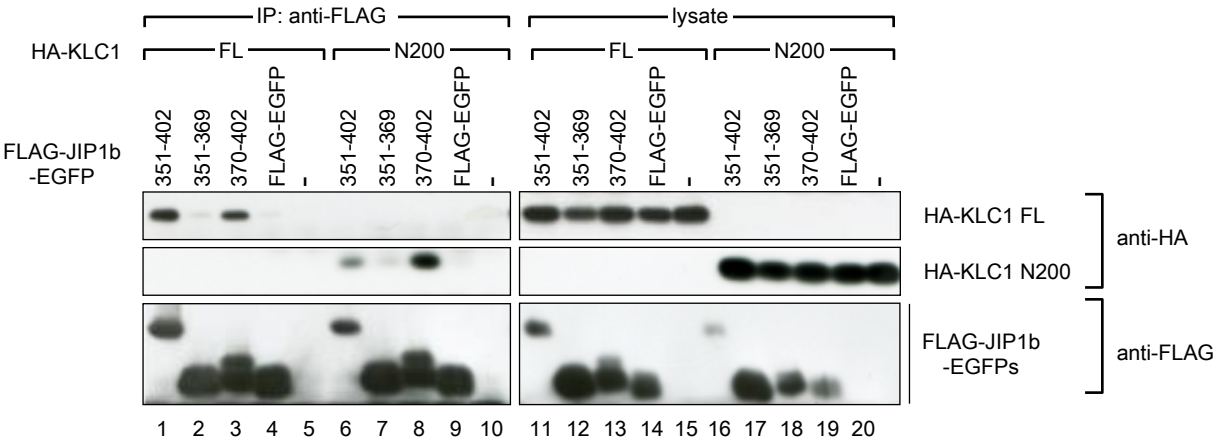


Figure S7

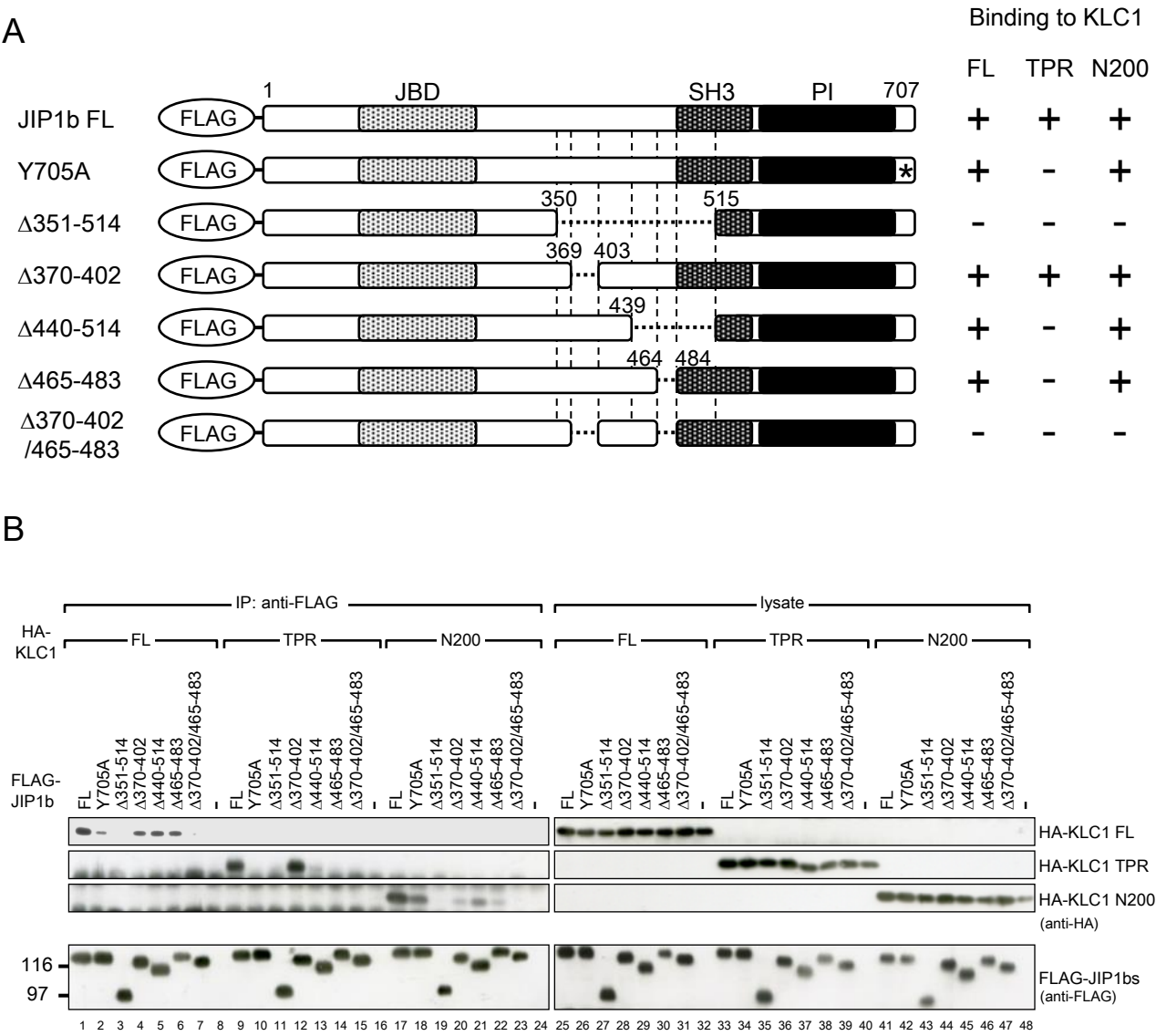
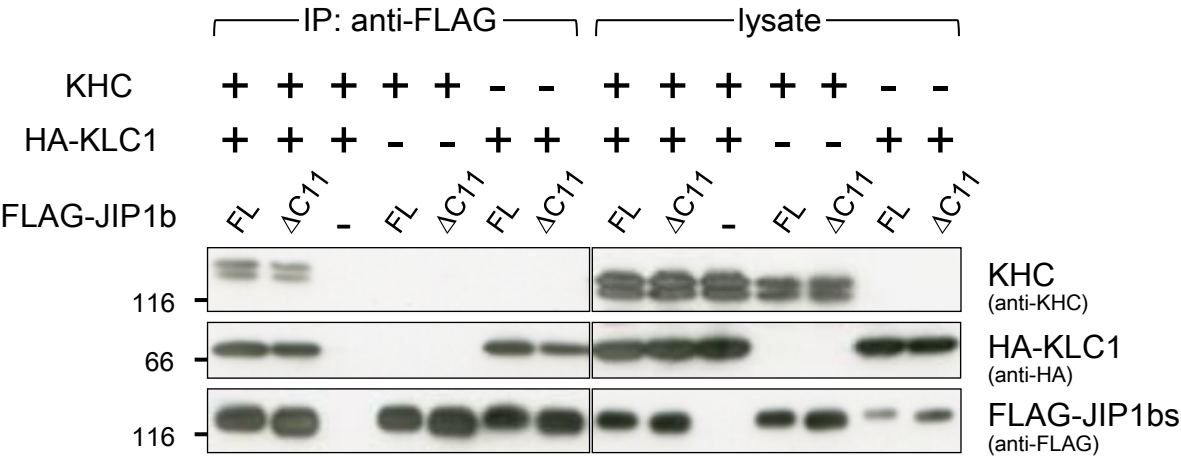


Figure S8

A



B

