Huynh and Vale, https://doi.org/10.1083/jcb.201703201

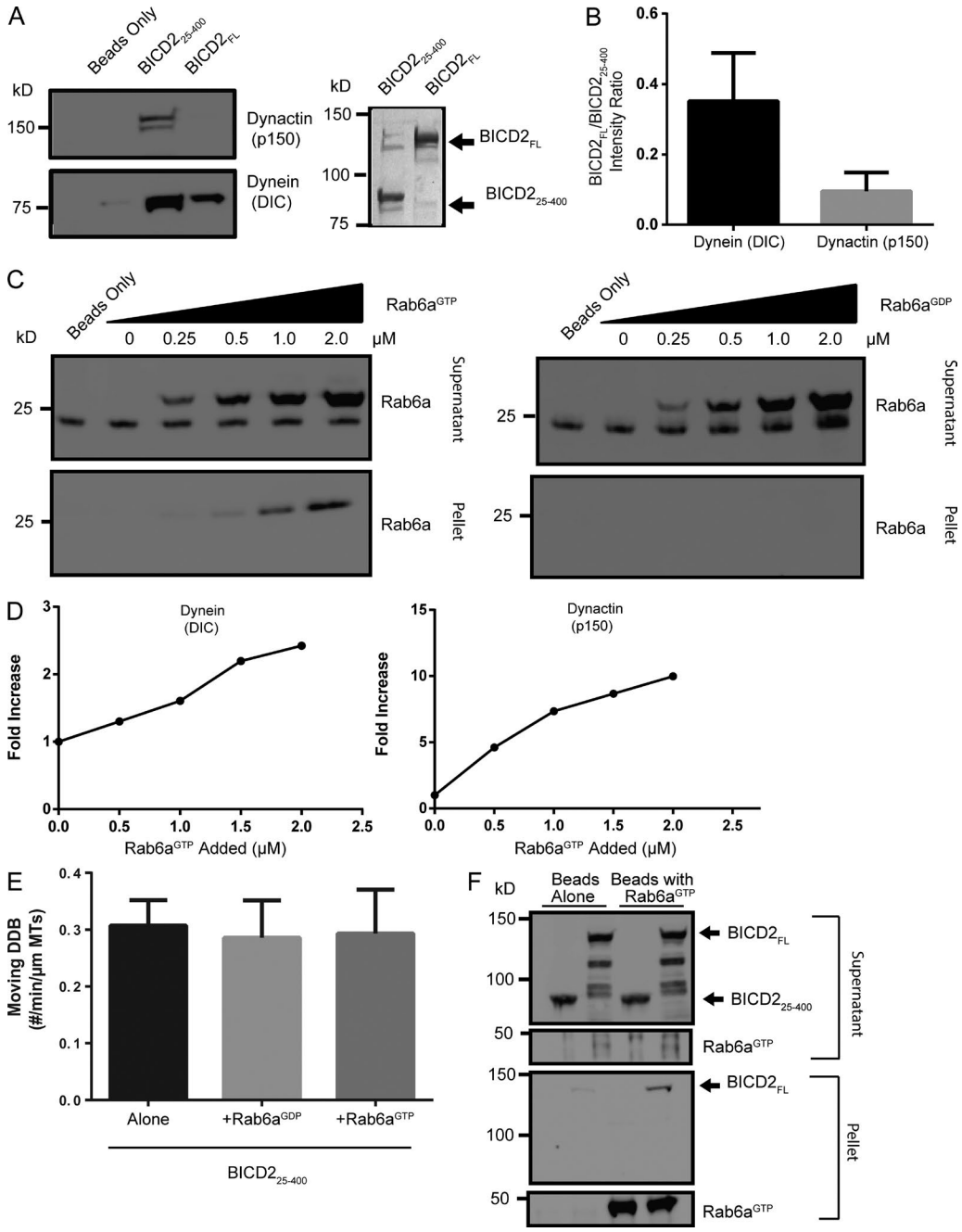


Figure S1. **$BICD2_{25-400}$ recruits more dynein compared with full-length.** (A) A comparison of the amounts of dynein and dynactin pulled down from porcine brain lysate between $BICD2_{25–400}$ and $BICD2_{FL}$ (B) Quantification of the pull-down; mean and SD from $n = 3$ independent experiments. (C) The supernatant and pellet fraction of the pull-downs from Fig. 1 B showing that $Rab6a^{GTP}$ but not $Rab6a^{GDP}$ binds to the BICD2 on beads. (D) Quantification of the Western blot shown in Fig. 1 B for the case of $Rab6a^{GTP}$ addition. Values are normalized to the lane in which no Rab6a is added. (E) Quantification of number of moving motors per micrometer of microtubule of $BICD2_{25–400}$ with no Rab6a added, $Rab6a^{GDP}$ added, or $Rab6a^{GTP}$ added; mean and SEM from $n = 3$ independent experiments (each experiment measuring a minimum of 30 microtubules). (F) GST–Rab6aGTP pull-down of either $BICD2_{25–400}$ or $BICD2_{FL}$.

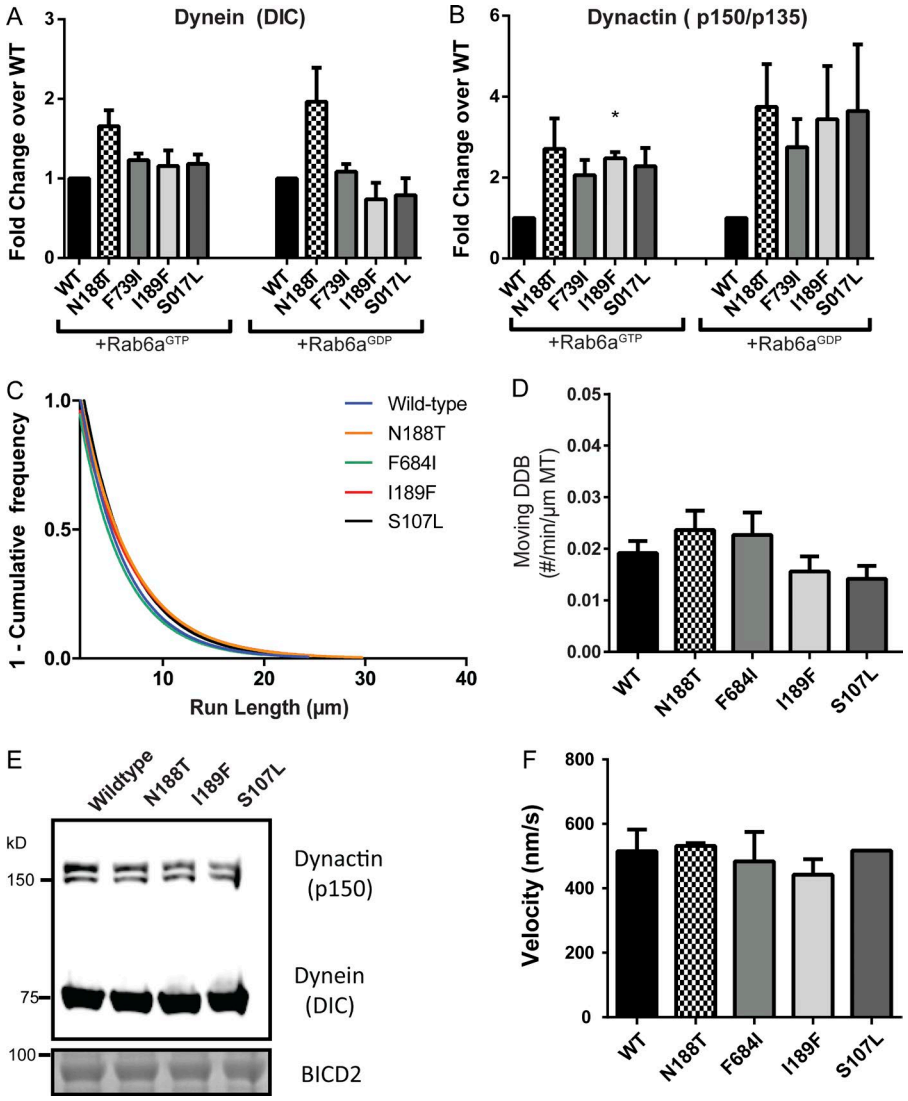


Figure S2. **N-terminal mutant constructs of BICD2 are comparable with WT.** (A and B) Quantification of the Western blots for Rab6a^{GTP} and Rab6a^{GDP} pull-downs from Fig. 2; mean and SEM from *n* = 3 independent experiments. For A, GTP addition mean and SEM are as follows: N188T: 1.66 ± 0.20, P = 0.082; F739I: 1.23 ± 0.082, P = 0.11; I189F: 1.16 ± 0.20, P = 0.51; and S107L: 1.18 ± 0.12, P = 0.26. GDP addition mean and SEM are as follows: N188T: 1.96 ± 0.42, P = 0.15; F739I: 1.08 ± 0.097, P = 0.47; I189F: 0.738 ± 0.21, P = 0.34; and S107L: 0.792 ± 0.21, P = 0.43. For (B), GTP addition mean and SEM are as follows: N188T: 2.71 ± 0.75, P = 0.15; F739I: 2.06 ± 0.375, P = 0.11; I189F: 2.48 ± 0.15, P = 0.011; and S107L: 2.28 ± 0.45, P = 0.10. GDP addition mean and SEM are as follows: N188T: 3.75 ± 1.06, P = 0.152; F739I: 2.75 ± 0.69, P = 0.13; I189F: 3.44 ± 1.3, P = 0.20; and S107L: 3.65 ± 1.6, P = 0.25. (C) Processivity data in the form of a one-cumulative frequency histogram for one replicate, comparing different full-length BICD2 constructs. (D) The motility assay for DDB + Rab6a^{GDP} is also shown; mean and SEM from *n* = 3 independent experiments. (E) A comparison of $BICD2_{25–400}$ for WT and appropriate mutant constructs in the porcine brain lysate pull-down assay. Shown are the Western blots of the pellet fractions for p150 and DIC and a Coomassie staining for BICD2. (F) Velocity measurements of liposomes shown in Fig. 4 D; mean and SEM from *n* = 3 independent experiments. Each experiment measured >100 liposomes. The error bar for S107L is too small to appear on the graph.

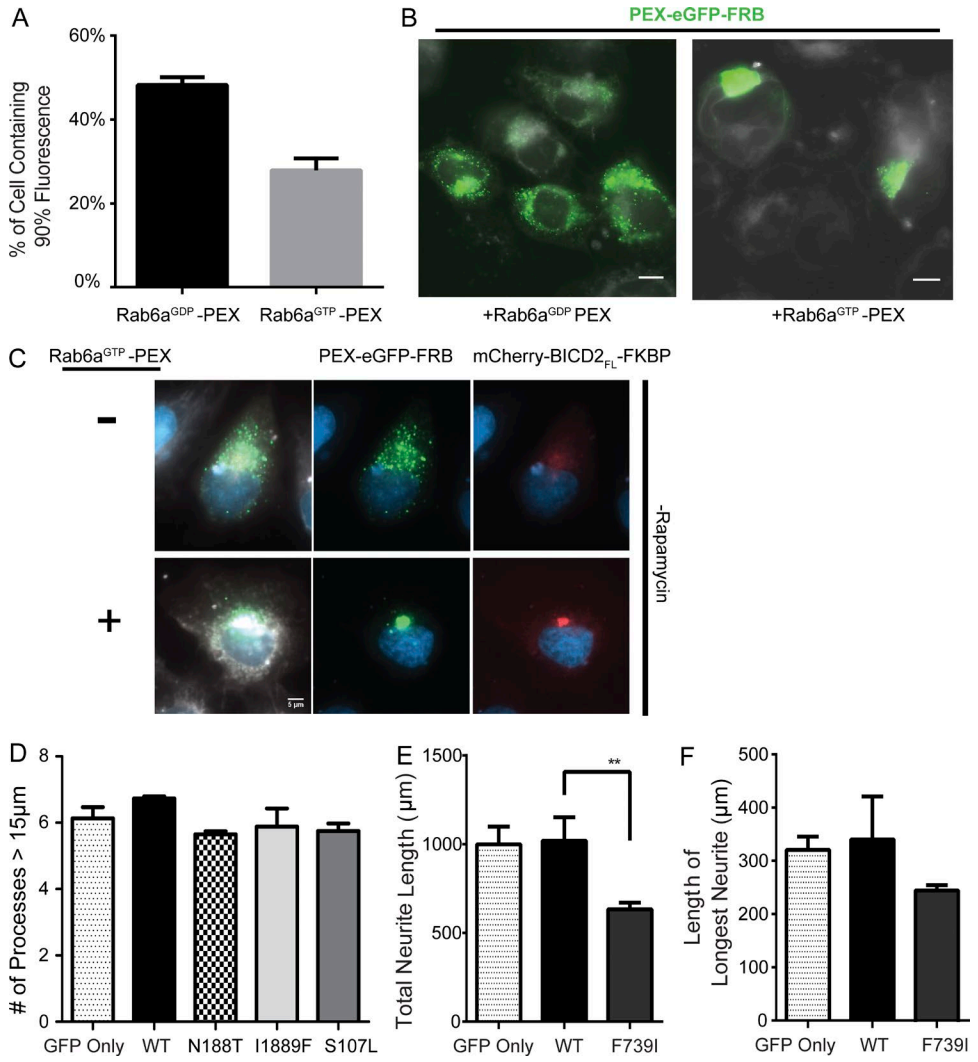
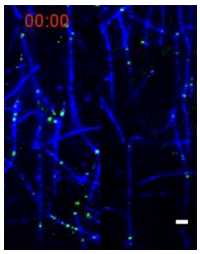
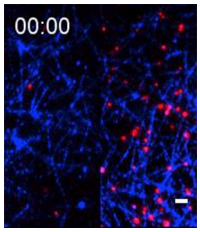


Figure S3. **Peroxisome-localized Rab6a^{GTP} is sufficient for clustering in cells.** (A) A comparison of peroxisome clustering when either the GDP or GTP of Rab6a is fused with the PEX sequence; mean and SEM from *n* = 2 independent experiments. Each experiment measured at least 30 cells. (B) Representative images of peroxisomes from A. The green color reflects PEX–eGFP–FRB, whereas the white is from a membrane dye, CellMask. Bar, 10 µm. (C) Example of BICD2$_{FL}$ localizing to the peroxisomes even in the absence of rapamycin. In the top row, cells were transfected with PEX–eGFP–FRB and mCherry–BICD2$_{FL}$. In the bottom row, the cells were additionally transfected with Rab6a^{GTP}–PEX as well. (D) Quantification of total number of processes (>15 µm long) emanating from the cell body of the neurons from Fig. 5 A; mean and SD from *n* = 3 independent experiments. (E) Total neurite length after 3 d of overexpression of BICD2$_{FL}$ constructs; mean and SD from *n* = 3 independent experiments (each experiment measuring 10–15 neurons for each construct); **, $P \leq 0.01$. (F) Axon length after 3 d of overexpression of BICD2$_{FL}$ constructs; mean and SD from *n* = 3 independent experiments (each experiment measuring 10–15 neurons for each construct).



Video 1. **Comparison of BICD2$_{25–400}$ versus BICD2$_{FL}$ DDB.** Video shows motility of either BICD2$_{25–400}$ (left) or BICD2$_{FL}$ (right) DDB complexes (green) on microtubules (blue). Bar, 2 µm. See Fig. 1 C for details.



Video 2. **Comparison of Rab6a^{GDP} and Rab6a^{GTP} liposome motility.** Video shows a comparison of Rab6aGDP (left) versus Rab6aGTP (right) liposomes (red) when incubated together with DDB$_{FL}$. Microtubules are shown in blue. Bar, 2 µm.