JCB

Supplemental material

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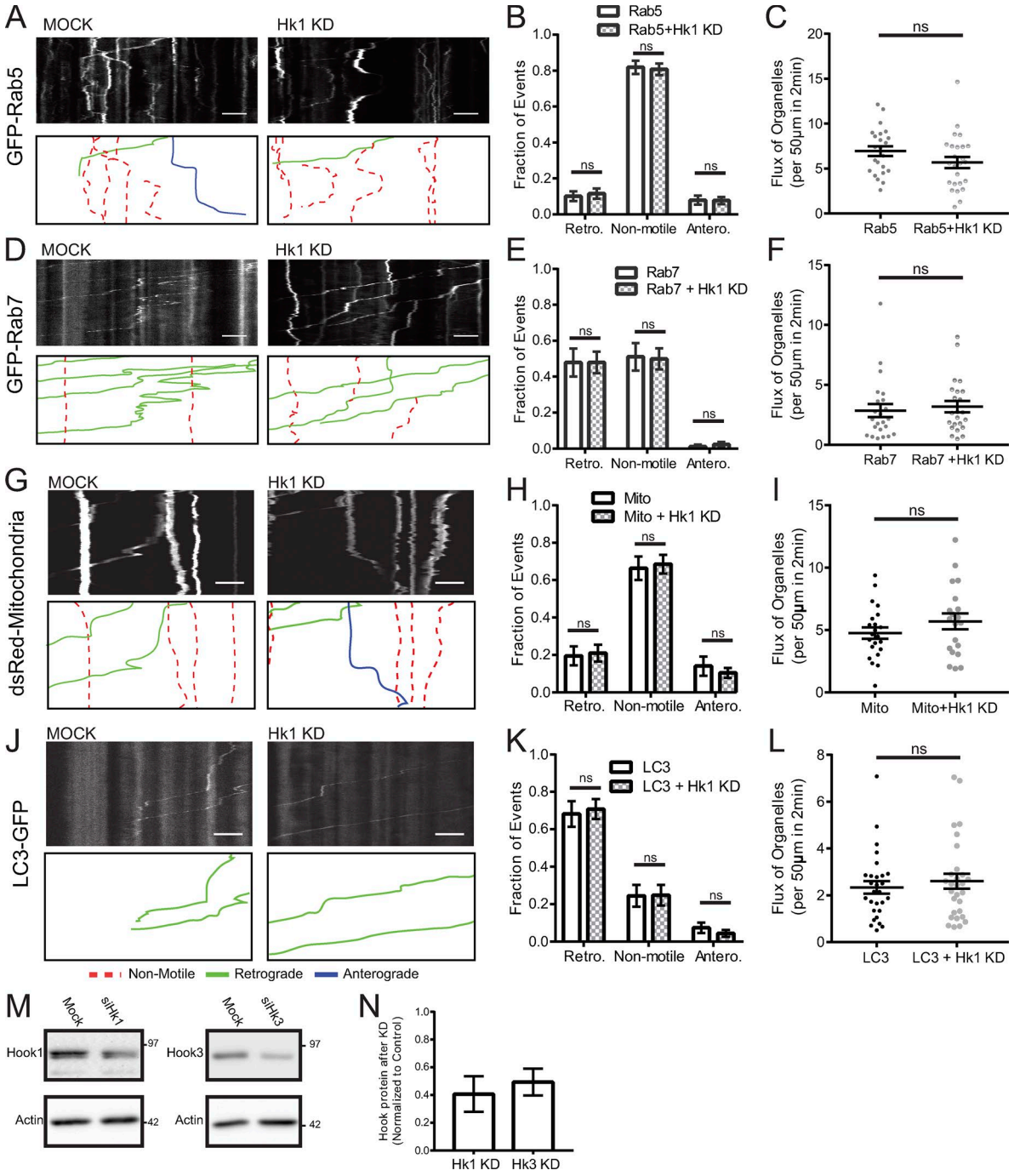


Figure S1. **Hook1 KD does not significantly change Rab5, Rab7, mitochondria, or autophagosome motility. (A)** Kymograph of Rab5-GFP in control or Hk1 KD neurons. Traced events below are color coded for ease of interpretation. **(B)** Motility fractioned into retrograde, anterograde, and nonmotile events per neuron. Bar graph shows mean ± SEM; two-way ANOVA (P > 0.9999). Rab5: *n* = 23 neurons; Rab5 + siHk1: *n* = 25 neurons. **(C)** Flux of Rab5 organelles in control or Hk1 KD neurons. Scatter plot shows mean ± SEM; unpaired *t* test (P = 0.1303). Rab5: *n* = 23 neurons; Rab5 + siHk1: *n* = 25 neurons. **(D)** Kymograph of Rab7-GFP in control or Hk1 KD neurons. Traced events below are color-coded for ease of interpretation. (A and D) Bars, 5 µm; 2 min total. **(E)** Motility fractioned into retrograde, anterograde, and nonmotile events per neuron. Bar graph shows mean ± SEM; two-way ANOVA (P > 0.9999). Rab7: *n* = 23 neurons; Rab7 + siHk1: *n* = 23 neurons. **(F)** Flux of Rab7 organelles in control or Hk1 KD neurons. Scatter plot shows mean ± SEM; unpaired *t* test (P = 0.6504). Rab7: n = 23 neurons; Rab7 + siHk1: n = 23 neurons. **(G)** Kymographs of mitochondria in control and Hook1 KD cells. Traced events below are color-coded for ease of interpretation. **(H)** Motility fractioned into retrograde, anterograde, and nonmotile events per neuron. Bar graph shows mean ± SEM; two-way ANOVA (P > 0.93). Mito: *n* = 22 neurons; Mito + siHk1: *n* = 20 neurons. **(I)** Flux of mitochondria in control or Hk1 KD neurons. Scatter plot shows mean ± SEM; unpaired *t* test (P = 0.2292). Mito: *n* = 22; Mito + siHk1: *n* = 20 neurons. **(J)** Kymographs of LC3-GFP in control and Hook1 KD cells. Traced events below are color-coded for ease of interpretation. (G and J) Bars, 5 µm; 1 min total. **(K)** Motility fractioned into retrograde, anterograde, and nonmotile events per neuron. Bar graph shows mean ± SEM; two-way ANOVA (P > 0.96). LC3: *n* = 27 neurons; LC3 + siHk1: *n* = 29 neurons. **(L)** Flux of LC3-GFP in control or Hk1 KD neurons. Scatter plot shows mean ± SEM; unpaired *t* test (P = 0.5335). LC3: *n* = 28 neurons; LC3 + siHk1: *n* = 29 neurons. **(M)** Western blots of Hook1 and Hook3 siRNA KD in PC12 cells. **(N)** Quantification of KD Western blots from three individual repeats. Bar graph shows mean ± SEM.

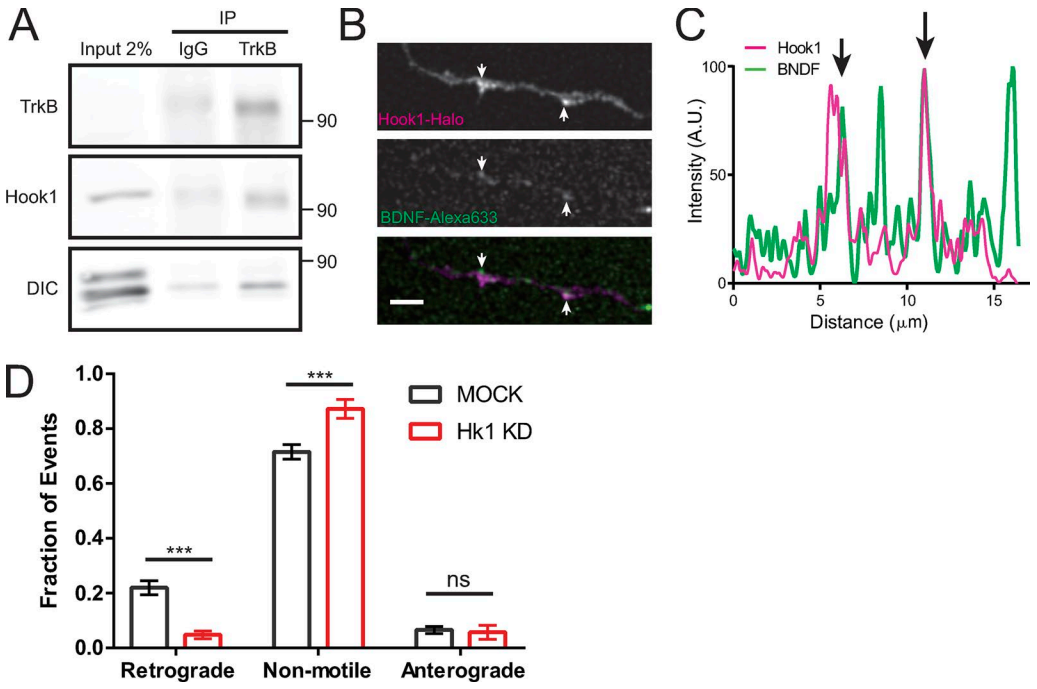
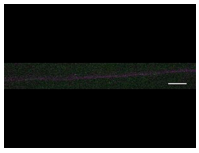
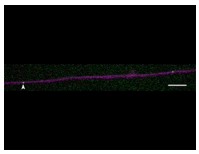


Figure S2. **Hook1 is present on TrkB–BDNF vesicles. (A)** Western blot of TrkB vesicle immunoisolation from mouse brain lysates. IP, immunoprecipitation. **(B)** Colocalization images of BDNF–Alexa Fluor 633 with Hook1-Halo in axon of hippocampal neuron. Arrows show Hook1 puncta colocalized with BDNF. Bar, 2 µm. **(C)** Line scan through axon in B. Arrows point to colocalized Hook1 and BDNF. **(D)** BDNF-Qdot motility fractioned into retrograde, anterograde, and nonmotile events per neuron. Bar graphs show mean ± SEM, two-way ANOVA (***, P < 0.0001; ns, P = 0.9931). Mock: *n* = 53 neurons; Hk1 KD: *n* = 44 neurons. Some data are repeated from Fig. 2 E.

Figure S3. **Sequence analysis of the Hook1 and Hook3 C terminus. (A)** Sequence alignments of Hook1 C termini from different species as noted. Blosum62 coloring; red asterisks indicate mutated residues. **(B)** Sequence alignment of Hook1 and Hook3 C termini. Blosum62 coloring; red asterisks indicate mutated residues.



Video 1. **BDNF-Qdot motility in control neurons grown in microfluidic chambers.** GFP fill in magenta and Qdots in green, soma to the left. Bar, 5 µm; four frames per second with playback at 7.5× real time.



Video 2. **BDNF-Qdot motility in Hk1 KD neurons grown in microfluidic chambers.** GFP fill in magenta and Qdots in green, soma to the left. Bar, 5 µm; four frames per second with playback at 7.5× real time.