Fig. S1. **NudE and p150 bind to alternatively spliced dynein IC isoforms.** *A.* Sequence alignment of both IC genes from *Rattus norvegicus* (46), showing GST-fusion constructs created in this study encoding the six IC isoforms. Alternatively spliced regions are highlighted in orange. *B.* HA-NudE or FLAG-CC1 pulled down using the GST-IC isoforms. The HA-NudE bands are distorted by the similar sized GST-IC constructs.

Fig. S2. **LC8 does not affect the NudE-dynein interaction.** *A.* Immunoblot of purified rat brain dynein pulled down with purified HA-NudE, which had been pre-incubated with recombinant LC8. Lane 1, HA-NudE-LC8 pull down - no dynein present; lane 2, HA-NudE - LC8 pull-down of purified dynein; Lane 3, HA-NudE pull-down of purified dynein; lane 4, beads alone. Endogenous LC8 (endo. LC8) in the purified dynein complex runs slightly faster than recombinant LC8 (rec. LC8). *B.* HA-NudE pulldown of dynein in the presence of excess LC8. Lanes 1-4, 0x, 5x, 10x, or 50x molar ratio (over NudE) of LC8; lane 5, beads alone.

Figure S1.

A

IC-1

```
Majority  MSDKSDLKAELERKKQRLAQIREEKKRKEEERKKKEADMQQKKEPVPDDSDLDRKRRETEALLQSIGISPEPP----------------LVPTPMSPSS
                         10        20        30        40        50        60        70        80        90       100
IC1A      MSDKSDLKAELERKKQRLAQIREEKKRKEEERKKKEADMQQKKEPVPDDSDLDRKRRETEALLQSIGISPEPPLVQPLHFLTWDTCYFHYLVPTPMSPSS 298
IC1B      MSDKSDLKAELERKKQRLAQIREEKKRKEEERKKKEADMQQKKEPVPDDSDLDRKRRETEALLQSIGISPEPP----------------LVPTPMSPSS 247
IC1C      MSDKSDLKAELERKKQRLAQIREEKKRKEEERKKKEADMQQKKEPVPDDSDLDRKRRETEALLQSIGISPEPP----------------LVPTPMSPSS 247

Majority  KSVSTPSEAGSQDDLGPLTRTLQWDTDPSVLQLQSDSELGRRLNKLGVSKVTQVDFLPREVVSYSKETQTPLATHQSEEDEEDEEM
                  110       120       130       140       150       160       170       180
IC1A      KSVSTPSEAGSQDDLGPLTRTLQWDTDPSVLQLQSDSELGRRLNKLGVSKVTQVDFLPREVVSYSKETQTPLATHQSEEDEEDEEM 556
IC1B      KSVSTPSEAGSQDDLGPLTRTLQWDTDPSVLQLQSDSELGRRLNKLGVSKVTQVDFLPREVVSYSKETQTPLATHQSEEDEEDEEM 505
IC1C      KSVSTPSEAGSQDDLGPLTR----------RLNKLGVSKVTQVDFLPREVVSYSKETQTPLATHQSEEDEEDEEM 442
```

IC-2

```
Majority  MSDKSELKAELERKKQRLAQIREEKKRKEEERKKKETDQKKEAAVSVQEESDLEKKRREAEALLQSMGLTTDSPIV------PPPMSPSSKSVSTPSEAG
                         10        20        30        40        50        60        70        80        90       100
IC2A      MSDKSELKAELERKKQRLAQIREEKKRKEEERKKKETDQKKEAAVSVQEESDLEKKRREAEALLQSMGLTTDSPIVFSEHWVPPPMSPSSKSVSTPSEAG 298
IC2B      MSDKSELKAELERKKQRLAQIREEKKRKEEERKKKETDQKKEAAVSVQEESDLEKKRREAEALLQSMGLTTDSPIV------PPPMSPSSKSVSTPSEAG 280
IC2C      MSDKSELKAELERKKQRLAQIREEKKRKEEERKKKETDQKKEAAVSVQEESDLEKKRREAEALLQSMGLTTDSPIV------PPPMSPSSKSVSTPSEAG 280

Majority  SQDSGDGAVGSRTLHWDTDPSALQLHSDSDLGRGPIKLGMAKITQVDFPPREIVTYTKETQTPVTAQPKEDEEEEDDV
                  110       120       130       140       150       160       170
IC2A      SQDSGDGAVGSRTLHWDTDPSALQLHSDSDLGRGPIKLGMAKITQVDFPPREIVTYTKETQTPVTAQPKEDEEEEDDV 532
IC2B      SQDSGDGAVGSRTLHWDTDPSALQLHSDSDLGRGPIKLGMAKITQVDFPPREIVTYTKETQTPVTAQPKEDEEEEDDV 514
IC2C      SQDSGDGAVGSR----------RGPIKLGMAKITQVDFPPREIVTYTKETQTPVTAQPKEDEEEEDDV 454
```

B

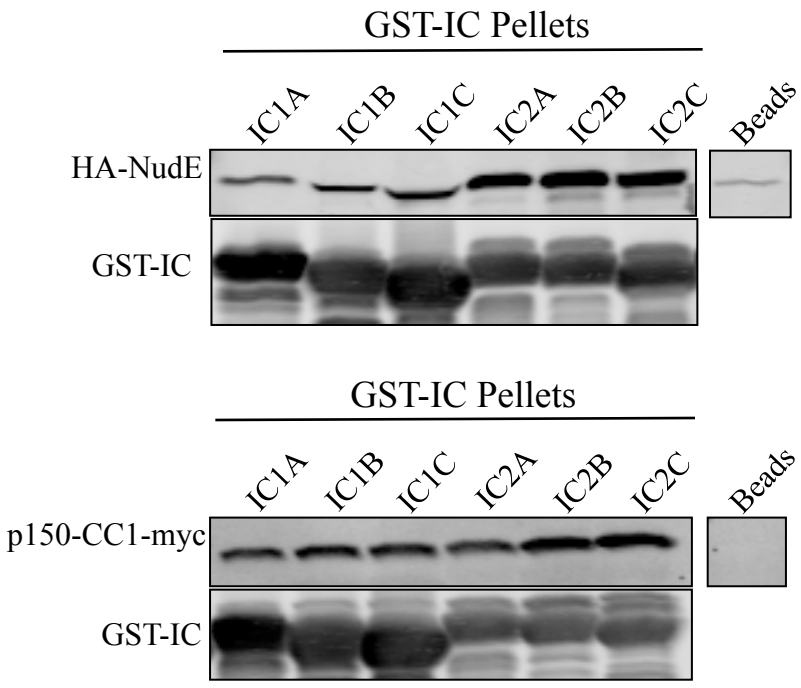
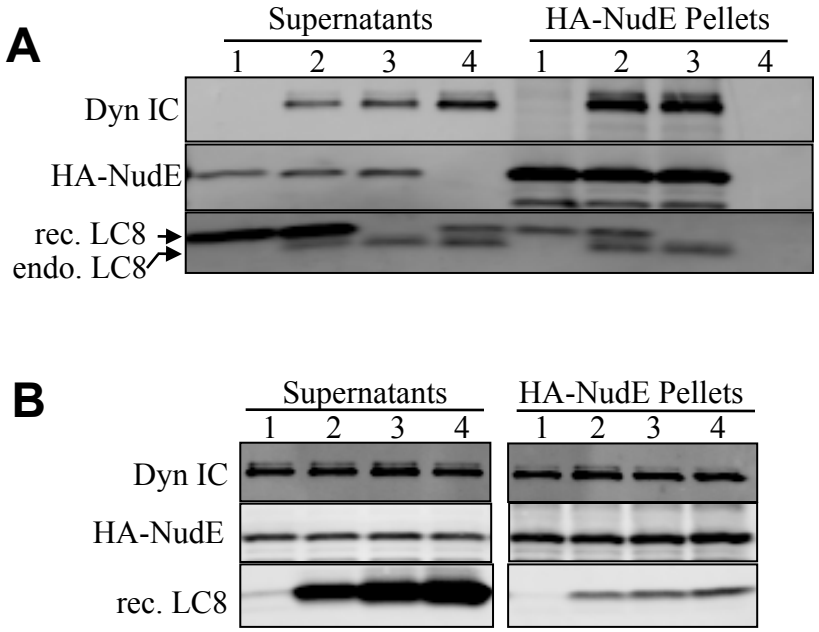


Figure S2.


A
Supernatants
HA-NudE Pellets
1 2 3 4 1 2 3 4
Dyn IC
HA-NudE
rec. LC8
endo. LC8
B
Supernatants
HA-NudE Pellets
1 2 3 4 1 2 3 4
Dyn IC
HA-NudE
rec. LC8