Supplemental Figure Legends

Figure S1. Verprolin-like and acidic regions of CARMIL family proteins. A) Alignment of a subset of CARMIL proteins with WH2 regions from various proteins, based on an alignment by Dominguez and colleagues (Chereau et al., 2005). Human CARMIL sequences are from accessions listed in Methods. B) Comparison of the acidic regions of CARMIL proteins. DNASTAR Protean was used to identify acidic regions and calculate pI and charge. Acidic regions of *Acanthamoeba* CARMIL (Acan125) and *Dictyostelium* CARMIL (p116) as identified in previous studies (Jung et al., 2001). In human CARMILs, the acidic region was generally not large and contiguous, but relatively small and dispersed. The human sequences were as follows: CARMIL1a: 877-886, 898-912, 936-955; CARMIL2b: 874-904, 926-945; and CARMIL3: 889-903.

Figure S2. Co-immunoprecipitation of CARMIL1 and CARMIL2 with various proteins. Immunoprecipitation was performed with Flag-tagged CARMIL1 or CARMIL2. The precipitates were analyzed by immunoblots with indicated antibodies. Trio has several isoforms and therefore appears as three bands, of Mr 250 kDa, 150 kDa and 98 kDa. p34 is a subunit of Arp2/3.

Figure S3. Efficacy and specificity of shRNAs targeting CARMIL1 and CARMIL2. Low-power fluorescence images of HEK293 cells expressing exogenous YFP fusions of CARMIL1 or CARMIL2, transfected with shRNA-expressing plasmids targeting CARMIL1 or CARMIL2. An RFP expression plasmid was co-transfected, to identify transfected cells. Similar results were obtained with HT-1080 cells.

Figure S4. YFP-CARMIL1 localization in migrating cells. Human SNB-19 cell and mouse B16-F1 cells expressing YFP-CARMIL1 were fixed and stained with phalloidin.

Figure S5. Dependence of CARMIL1 localization on Arp2/3 complex. A) Level of the p34 subunit of Arp2/3 complex, by immunoblot, in cells transfected with siRNA targeting the p20 subunit of Arp2/3 complex. Negative controls include siRNA targeting CARMIL2 (C2siRNA), siRNA with a scrambled sequence, and mock transfection (None). B) Loss of lamellipodial actin at the leading edge after knockdown of p20. Cells were stained with anti-p34, anti-cortactin, and fluorescent phalloidin. C) YFP-CARMIL1 localization in Arp2/3-knockdown cells.

Cells as in (B) transfected with p20siRNA and co-transfect with YFP-CARMIL1. The presence of YFP-CARMIL1 fluorescence identifies transfected cells.

Figure S6. CARMIL2 colocalization with vimentin filaments. A) HT-1080 cells expressing YFP-CARMIL2 were extracted, fixed and stained with anti-vimentin Ab. Arrowheads point to regions enlarged in insets. B) CARMIL2 collapses with vimentin filaments when microtubules are depolymerized with nocodazole. HT-1080 cells expressing YFP-CARMIL2 were treated with 5 μM nocodazole for 1hr, then nocodazole was washed out for 1hr. Cells were stained with anti-vimentin and anti-tubulin Abs. Arrows point to the perinuclear area, which contains microtubules before nocodazole and after washout. Arrowheads point to the alignment of YFP-CARMIL2 with vimentin.

Figure S7. Abnormal cell protrusions induced by overexpression of CARMIL1. HT-1080 cells expressing YFP-CARMIL1 were fixed and stained with antibodies against VASP, CP, Arp2/3 and cortactin. Cortical regions rich in YFP-CARMIL1 stain strongly for CP, Arp2/3 and cortactin, which are markers of branched networks of actin filaments, but not for VASP, a marker of filopodial bundles of filaments. Scale bar, 20 μm.

Supplemental Movies

Movies 1 - 3. Effects of knockdown of CARMIL1 or CARMIL2 on cell migration during wound healing assay. Phase-contrast time-lapse movies of HT-1080 cells on fibronectin. Movie 1 shows control scrambled shRNA cells, Movie 2 shows CARMIL1-knockdown cells, and Movie 3 shows CARMIL2-knockdown cells. Images were captured every 1.5 min for 2 hr, and the movies are designed to play at 15 frames per sec. GFP fluorescence indicates transfected cells.

Movies 4 - 7. Effects of knockdown of CARMIL1 or CARMIL2 on cell polarity during random migration. Phase-contrast time-lapse movies of HT-1080 cells on fibronectin. Movie 4 is a cell expressing control scrambled shRNA, Movie 5 is a CARMIL1-knockdown cell, and Movies 6 and 7 are CARMIL2-knockdown cells. Images were captured every 2 min for 2 hr, and the movies are designed to play at 6 frames per sec.

Movies 8 - 10. Effects of knockdown of CARMIL1 or CARMIL2 on lamellipodial dynamics. Phase-contrast time-lapse movies of HT-1080 cells on fibronectin. Movie 8 is a cell expressing control scrambled shRNA, Movie 9 is a CARMIL1-knockdown cell, and Movie 10 is a CARMIL2-knockdown cell. Images were captured every 1 sec for 5 min, and the movies are designed to play at 6 frames per sec.

Movies 11 - 13. Effects of knockdown of CARMIL1 or CARMIL2 on cell spreading. Phase-contrast time-lapse movies of newly plated HT-1080 cells, on a fibronectin-coated surface. Movie 11 is a cell expressing control scrambled shRNA, Movie 12 is a CARMIL1-knockdown cell, and Movie 13 is a CARMIL2-knockdown cell. In each case, the arrow indicates the transfected cell of interest; other cells in the field were not transfected. Images were captured every 30 sec for 30 min, and the movies are designed to play at 2 frames per sec.

Movies 14 - 15. Localization of YFP-CARMIL1 vs YFP-CARMIL2 in HT1080 cells undergoing macropinocytosis, by phase-contrast and fluorescence time-lapse movies. Movie14 shows YFP-CARMIL1 targeted to newly formed macropinosomes, while Movie15 shows the lack of such targeting in a YFP-CARMIL2 cell.

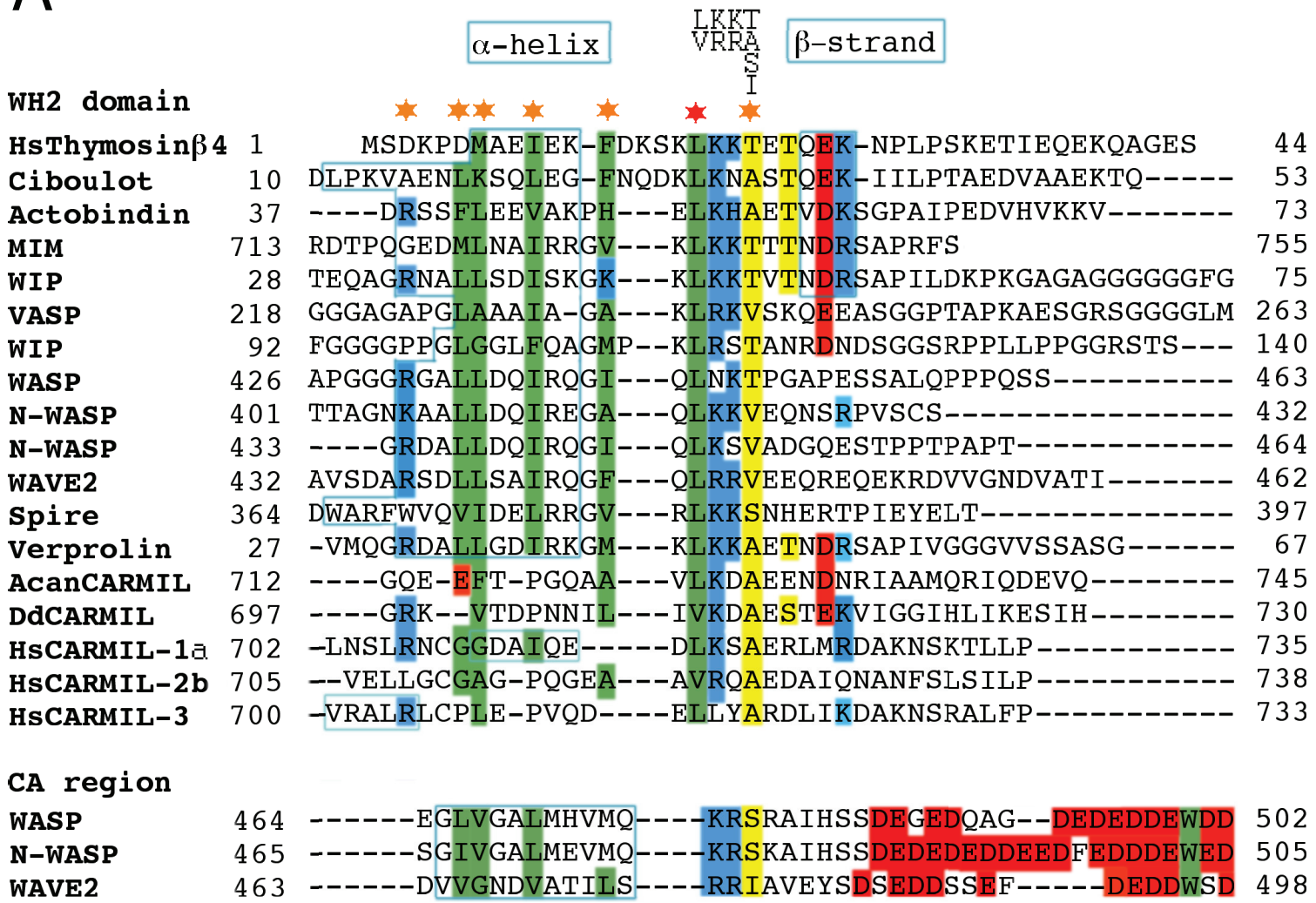
Supplemental Tables

Supplemental Table 1. Oligonucleotides for PCR amplification of HsFL-CARMIL1, HsFL-CARMIL2, fragments of Hs CARMIL family, capping protein alpha1, alpha2 and beta-actin. For is Forward, Rev is reverse, FL is full-length, and Frag is fragment.

Name	Sequence
HsCARMIL1 FL For	GCCGAATTCAATGACCGAGGAGAGCTCTGACGTTC
HsCARMIL1 FL Rev	GCCGGATCCTTACACAAAAATAAACTCTTTTTC
HsCARMIL2 FL For	AAGCTTCAATGGCCCAGACCCCCGACGGCATCTC
HsCARMIL2 FL Rev	GTCGACTCAGGGATTGGGGCCGCCGCCTCTTT
HsCARMIL1 Frag For	AAGAAATAGGGAAGGTGGAACGG
HsCARMIL1 Frag Rev	AATGTCATCAGGCCGTGAGGCGG
HsCARMIL2 Frag For	CCTTTGAACAGCGGGTAAAGTA
HsCARMIL2 Frag Rev	TCGGTTCCGAGGCCAGATCCTA
HsCARMIL3 Frag For	CCCAAAGCACCAAACCAAGCTTCAG
HsCARMIL3 Frag Rev	TGTTGACTCCTGGAGCTGCATCTC
CPalpha1 Frag For	GCAGCCAGAGGATGTGGATGGA
CPalpha1 Frag Rev	GATACTCATTTTCTGCACTCTCTATG
CPalpha2 Frag For	CCATTGAATCCTGGAGAACTTCAGTA
CPalpha2 Frag Rev	AATGCCAACCACCTGTGTGGTGGAA
beta-actin Frag For	TCACCCACACTGTGCCCATCTA
beta-actin Frag Rev	TACTCCTGCTTGCTATCCACA

Fig.S1, Liang et al.

A



B

	Charge	PI
Acan125	-16.06	3.09
DdCARMIL	-9.77	3.98
HsCARMIL-1 a	-7.75	4.65
HsCARMIL-2 b	-16.06	4.05
HsCARMIL-3	-6.91	3.37

Fig. S2, Liang et al.

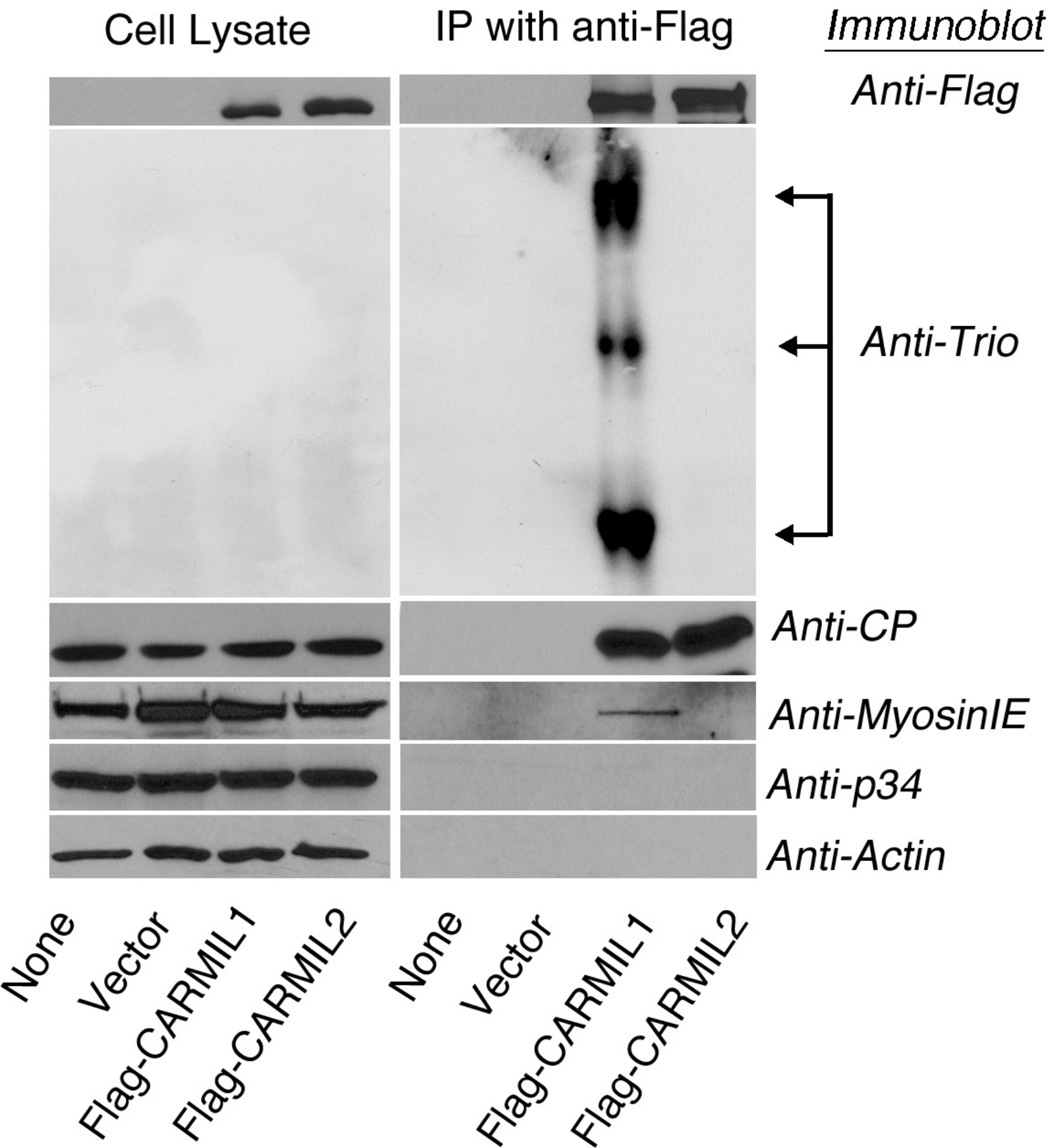


Fig. S3, Liang et al

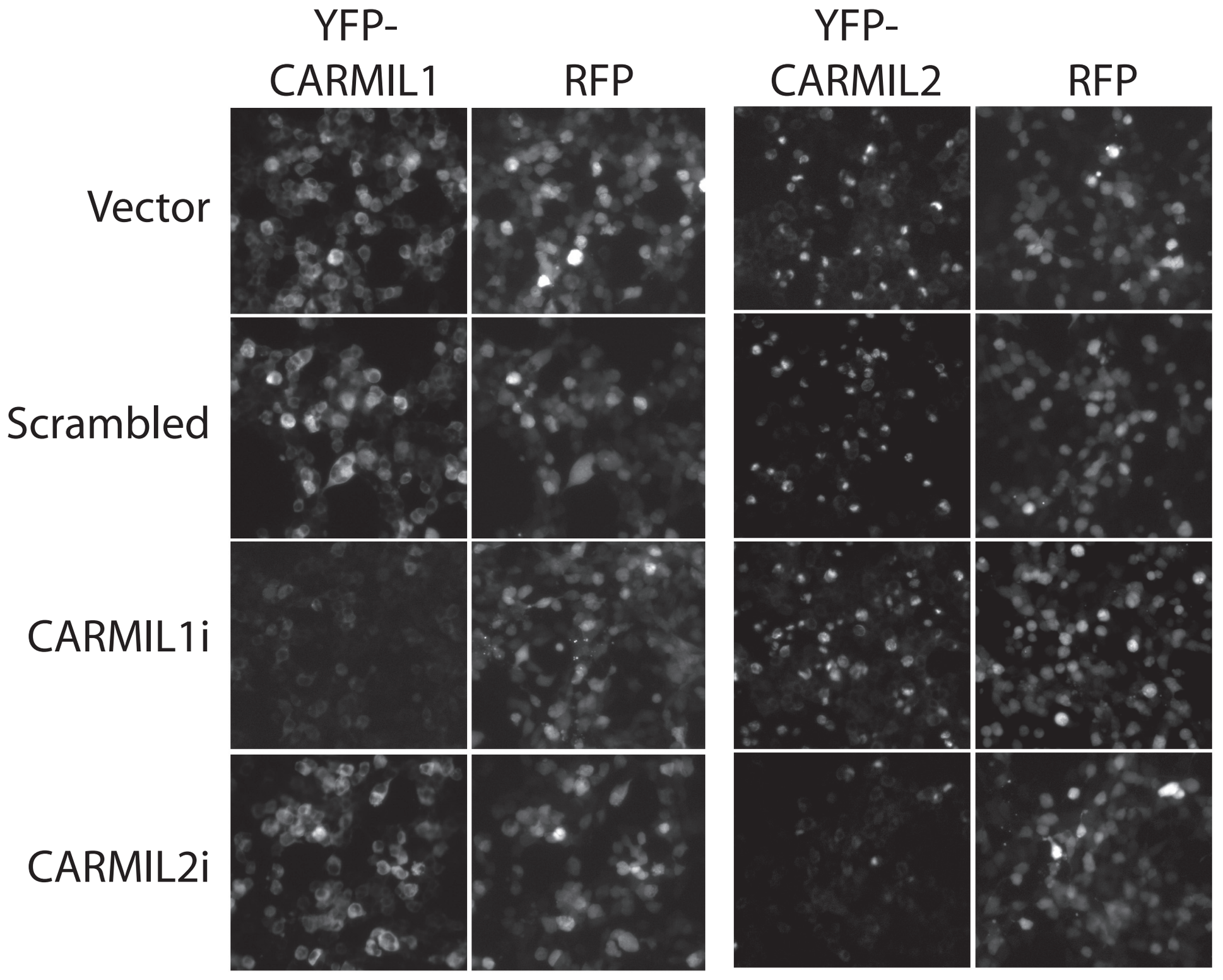


Fig.S4, Liang et al.

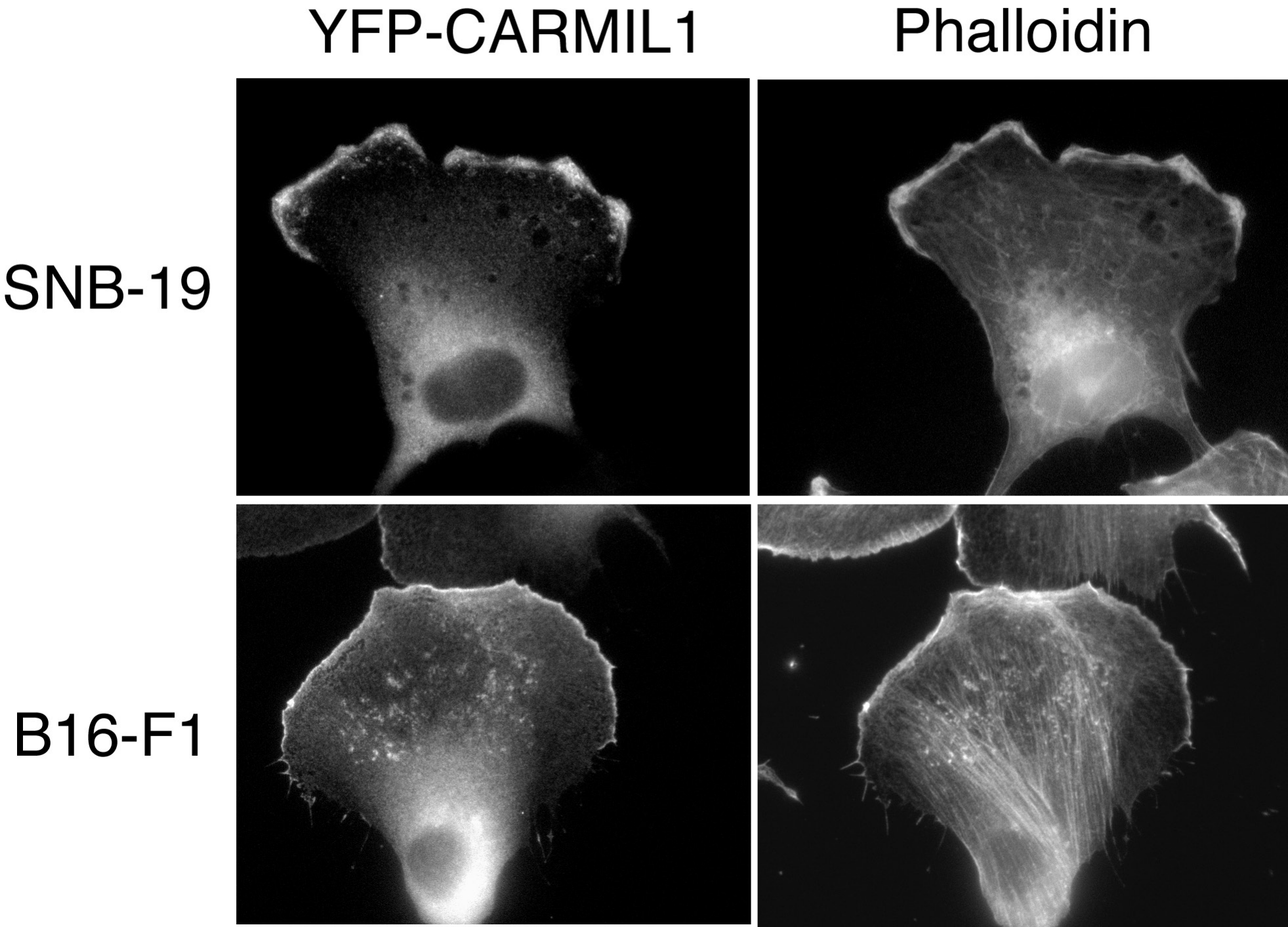
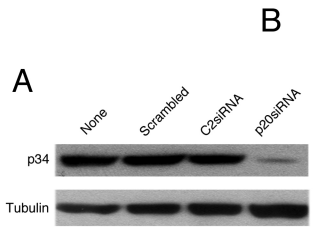
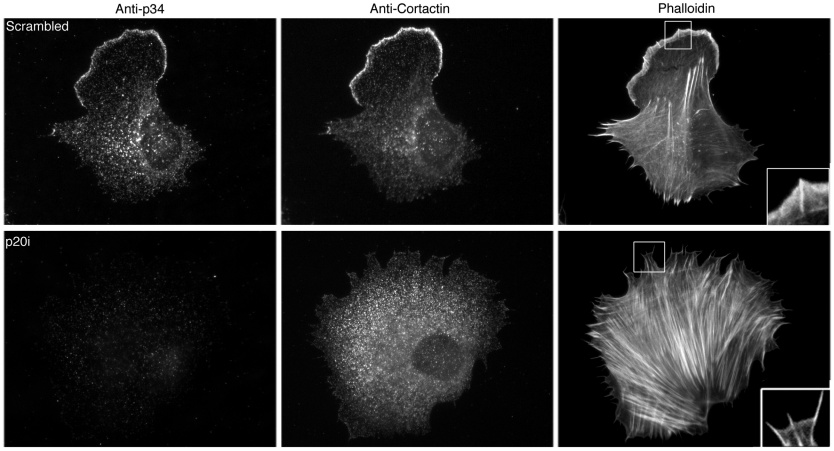


Fig. S5, Liang et al.





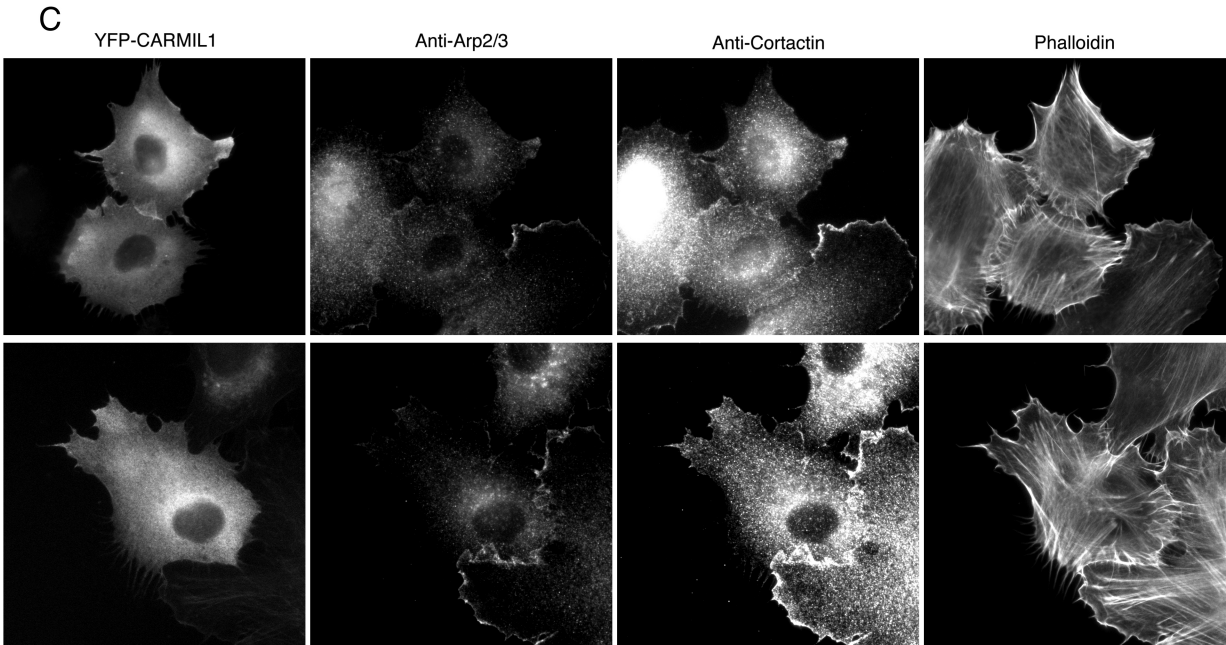


Fig. S6, Liang et al.

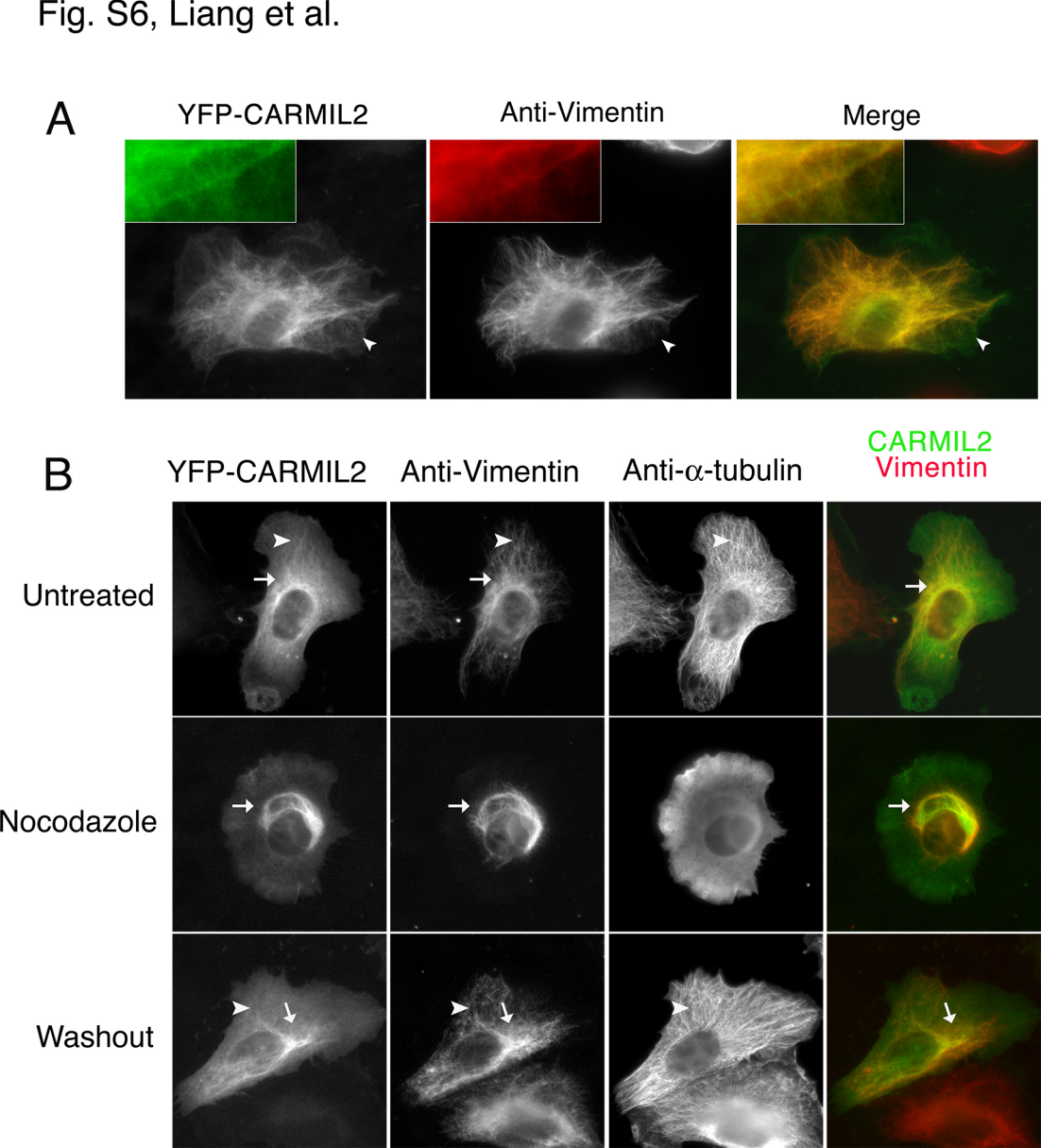


Fig. S7, Liang et al.