Supplemental Materials

Molecular Biology of the Cell

Bird and Barzik et al.

Supplementary Figure 1

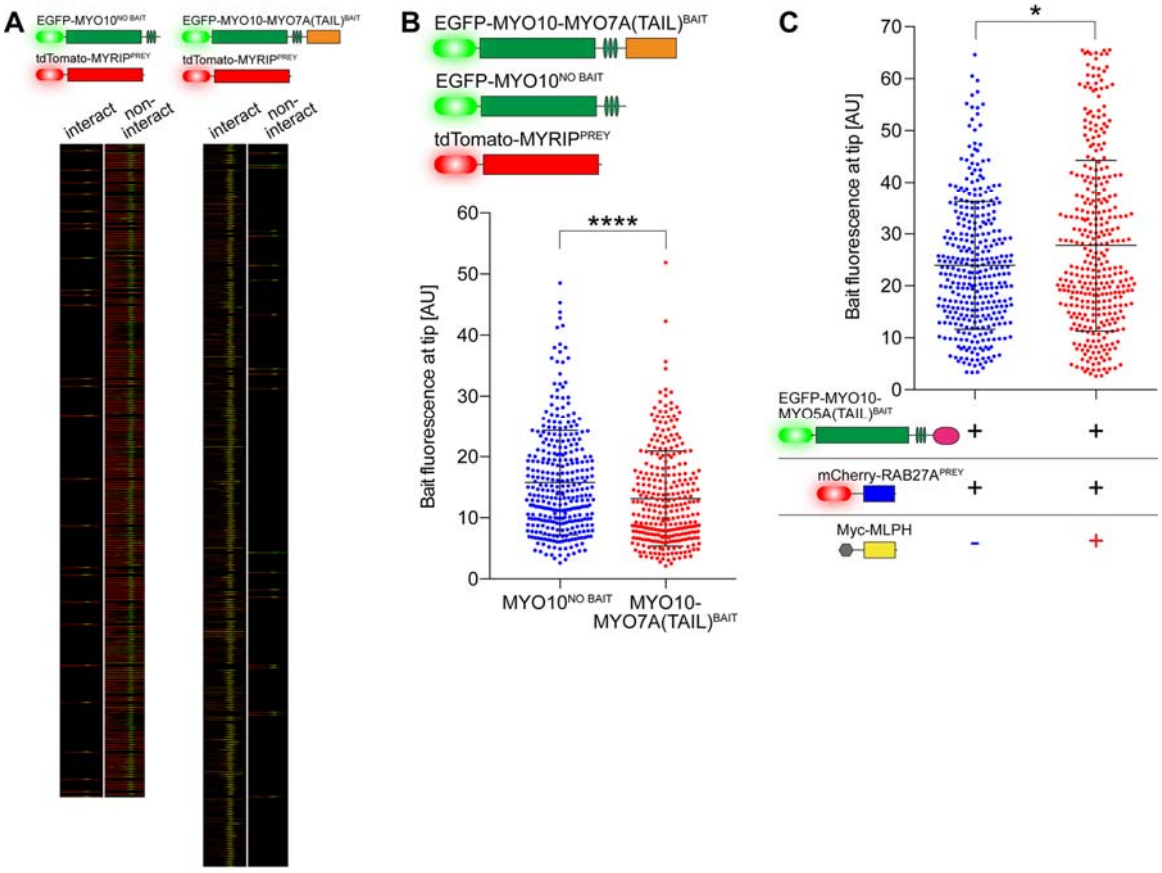

Figure S1. Fusion of bait molecules to MYO10 does not interfere with filopodia trafficking.

(A) Graphical summary of spatial correlation analysis from Fig. 3A,C. Bait (green) and prey (red) line scans for each filopodium are remapped along a straight line (aligned to the filopodium tip). Filopodia classified with bait-prey correlation ('Interact') are partitioned to the left column, and to the right ('Non-interact') otherwise. (B) Comparison of bait fluorescence intensities at filopodia tips of Sf9 cells expressing $MYO10^{NO\ BAIT}$ + $MYRIP^{PREY}$, or MYO10-$MYO7A(TAIL)^{BAIT}$ + $MYRIP^{PREY}$. Though bait fluorescence is statistically different (Mann-Whitney *U* test), the effect size is minimal indicating the MYO10 motor domain traffics equally, with (red) or without (blue) a bait molecule attached. Data are mean ± SD, from 3 independent determinations. (C) Bait intensity of MYO10-$MYO5A^{BAIT}$ at Sf9 filopodia tips in the absence (blue) or presence (red) of myc-MLPH. Again, although the bait fluorescence is statistically different (Mann-Whitney *U*-test), the effect size is small, indicating no substantial difference in bait molecule trafficking. Data are mean ± SD, from 3 independent determinations. * = $P < 0.05$; **** = $P < 0.0001$.

Supplementary Figure 2

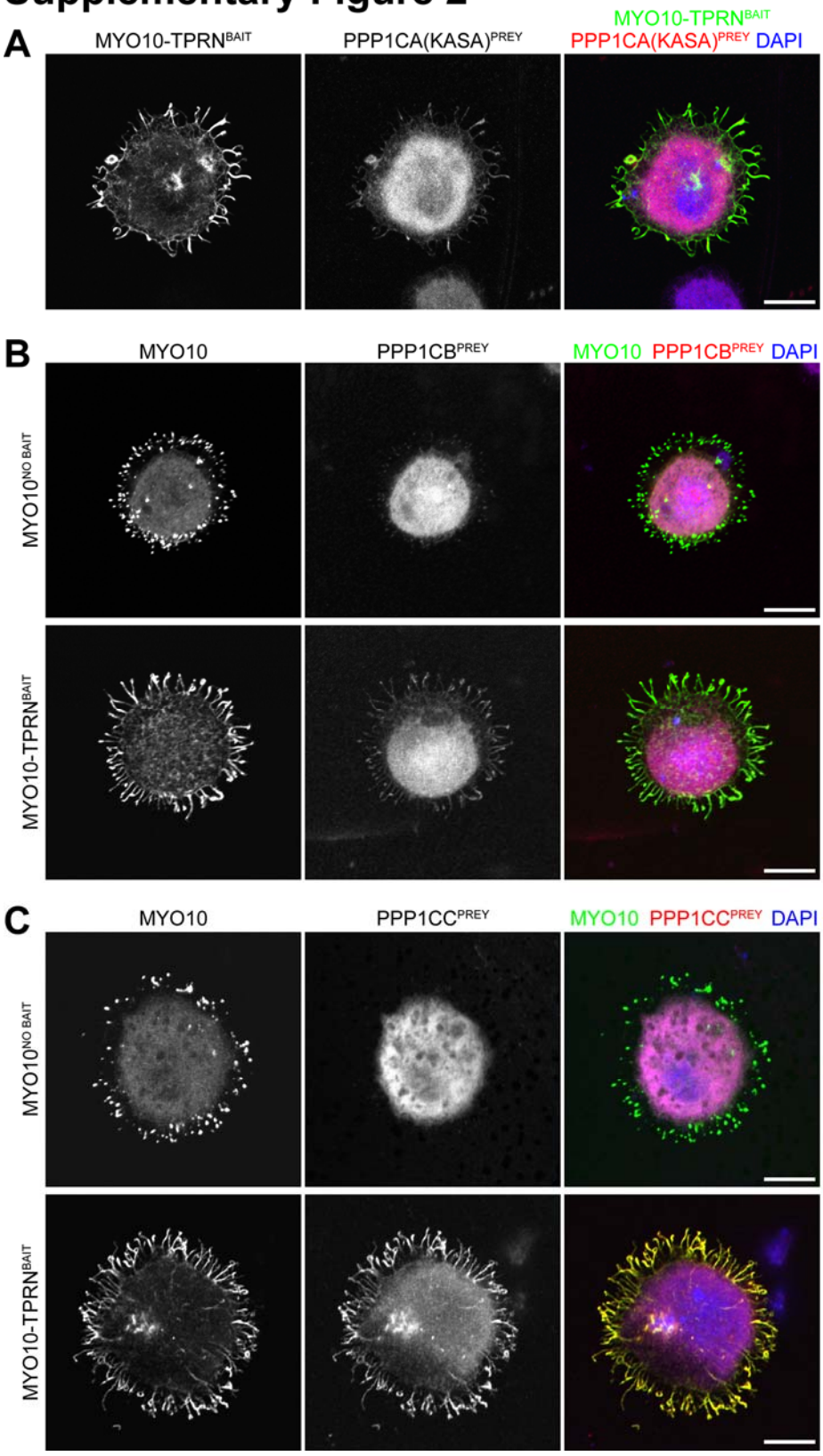

Figure S2. Taperin selectively interacts with the catalytic subunits of PP1.
(A) Two point mutations in a conserved motif (KISF → KASA) within taperin disrupt its interaction with PPP1CA. (B) PPP1CB does not significantly interact with taperin.
(C) Taperin interacts with PPP1CC. All images are from Sf9 cells, with taperin (green), PPP1C isozymes (red) and nuclei labeled with DAPI (blue). Scale bars, 10 μm.

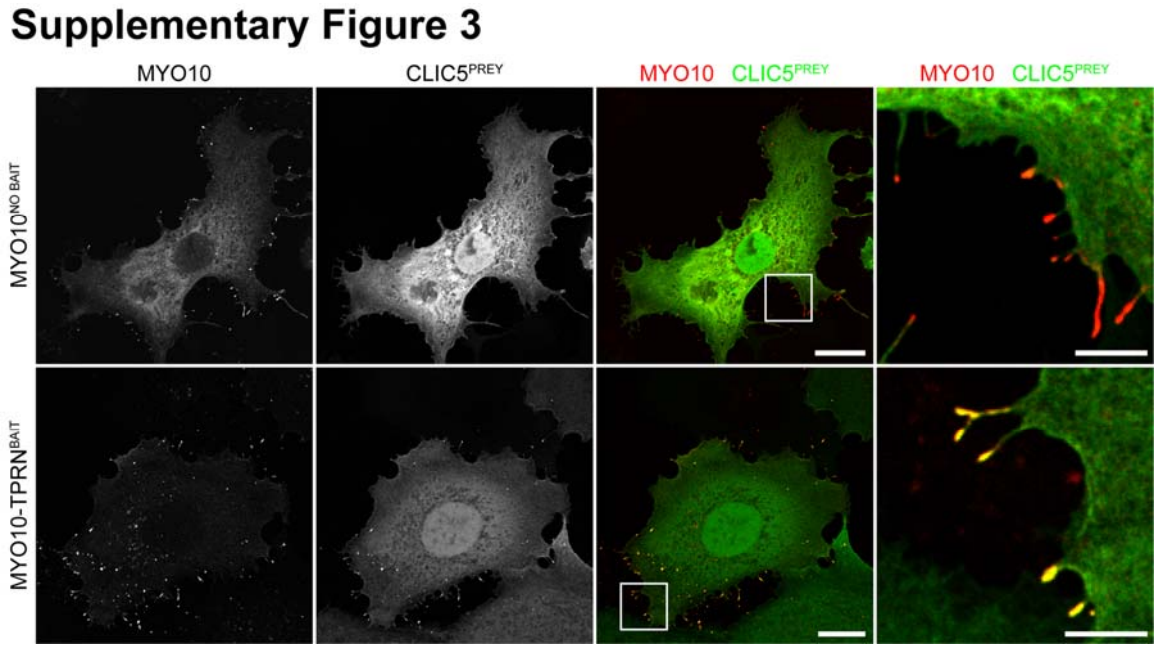

Figure S3. Taperin interacts with CLIC5.
$CLIC5^{PREY}$ (green) was co-expressed with either $MYO10^{NO\ BAIT}$ (control, red, top row) or $MYO10\text{-}TPRN^{BAIT}$ (query, red, bottom row). Boxed insets are shown magnified (right column). Scale bars, 20 μm; 5 μm in magnified insets.

SUPPLEMENTAL MATERIALS

Supplemental Table S1. Plasmids used in this study

Expression construct	Vector (Source)	Accession #	Encoded amino acids
Mammalian expression constructs			
pEGFP-MYO10	pEGFP-C2 (Clontech)	NP_062345	1-2062
pEGFP-MYO10-HMM ($MYO10^{NO\ BAIT}$)	pEGFP-C3 (Clontech)	NP_062345	1-941
pcDNA3.1(+)-MYO10-HMM-Nanotrap ($MYO10^{NANOTRAP}$)	pcDNA3.1(+) (ThermoFisher Scientific)	MYO10: NP_062345; Nanotrap: N/A (Rothbauer et al., 2008)	MYO10: 1-941, Nanotrap: 1-118
pmCherry-MYO10-HMM ($MYO10^{NO\ BAIT}$)	pmCherry-C1 (Clontech)	NP_062345	1-941
pEGFP-MYO10-MYO7A(TAIL)^{BAIT}	pEGFP-MYO10-HMM (this study)	AY821853	870-2166
pmCherry-MYRIP^{BAIT}	pmCherry-C1 (Clontech)	NP_653140	1-856
pmCherry-MYO10-HMM ($MYO10^{NO\ BAIT}$)	pmCherry-MYO10-HMM (this study)	NP_062345	1-941
pEGFP-MYO7A-TAIL (EGFP-MYO7A(TAIL)^{BAIT})	pEGFP-C2 (Clontech)	AY821853	870-2166
pmCherry-MYO10-TPRN^{BAIT}	pmCherry-MYO10-HMM (this study)	NP_780495	1-749
pEGFP-CLIC4^{PREY}	pEGFP-C2 (Clontech)	NP_038913	1-253
pEGFP-CLIC5^{PREY}	pEGFP-C2 (Clontech)	NP_766209	1-251
pEGFP-MYO10-TPRN^{BAIT}	pEGFP-C3 (Clontech)	NP_780495	1-749
pmCherry-CHD7	pmCherry-C1 (Clontech)	NP_060250	1-2997
Insect expression constructs			
pIZ-EGFP	pIZ/V5-His (Invitrogen)		

pIZ-mCherry	pIZ/V5-His (Invitrogen)		
pIZ-tdTomato	pIZ/V5-His (Invitrogen)		
pIZ-myc	pIZ/V5-His (Invitrogen)		
pIZ-EGFP-MYO10-HMM (MYO10$^{NO\ BAIT}$)	pIZ-EGFP (this study)	NP_062345	1-941
pIZ-EGFP-MYO10-MYO7A(TAIL)^{BAIT}	pIZ-EGFP-MYO10-HMM (this study)	AY821853	870-2166
pIZ-tdTomato-MYRIP^{BAIT}	pIZ-tdTomato (this study)	NP_653140	1-856
pIZ-EGFP-MYO10-MYO5A(TAIL)^{BAIT}	pIZ-EGFP-MYO10-HMM (this study)	NP_034994	931-1853
pIZ-mCherry-Rab27a^{PREY}	pIZ/V5-His (Invitrogen)	NP_001288161	1-221
pIZ-myc-MLPH	pIZ-myc (this study)	NP_443748	1-590
pIZ-EGFP-MYO10-TPRN^{BAIT}	pIZ-EGFP-MYO10-HMM (this study)	NP_780495	1-749
pIZ-EGFP-MYO10-TPRN(KASA)^{BAIT}	pIZ-EGFP-MYO10-HMM (this study)	NP_780495	1-749, with the following point mutations: I625A, F627A
pIZ-mCherry-PPP1CA^{PREY}	pIZ-mCherry (this study)	NP_114074	1-330
pIZ-mCherry-PPP1CB^{PREY}	pIZ-mCherry (this study)	NP_766295	1-327
pIZ-mCherry-PPP1CC^{PREY}	pIZ-mCherry (this study)	NP_038664	1-323
pIZ-mCherry-CHD4(561-936)^{PREY}	pIZ-mCherry (this study)	NP_666091	561-936
pIZ-mCherry-CHD4(DEXDc)^{PREY}	pIZ-mCherry (this study)	NP_666091	715-906
pIZ-EGFP-MYO10-TPRN(1-260)^{BAIT}	pIZ-EGFP-MYO10-HMM (this study)	NP_780495	1-260
pIZ-EGFP-MYO10-TPRN(261-622)^{BAIT}	pIZ-EGFP-MYO10-HMM (this study)	NP_780495	261-622
pIZ-EGFP-MYO10-TPRN(623-749)^{BAIT}	pIZ-EGFP-MYO10-HMM (this study)	NP_780495	623-749

pIZ-mCherry-CHD1(DEXDc)^{PREY}	pIZ-mCherry (this study)	NP_031716	475-672
pIZ-mCherry-CHD2(DEXDc)^{PREY}	pIZ-mCherry (this study)	NP_001074814	480-677
pIZ-mCherry-CHD3(DEXDc)^{PREY}	pIZ-mCherry (this study)	NP_666131	735-1001
pIZ-mCherry-CHD7(DEXDc)^{PREY}	pIZ-mCherry (this study)	NP_001264078	955-1155

Table S2. Yeast 2-hybrid interactions identified with taperin
Results from mouse inner ear Y2H screen

Gene name	# of clones	Confirmed by NanoSPD?
Chromodomain helicase DNA binding protein 4 (*Chd4*)	16	**Yes**
Protein phosphatase 1 catalytic subunit alpha (*Ppp1ca*)	5	**Yes**
Chromodomain helicase DNA binding protein 3 (*Chd3*)	3	**Yes**
Chloride intracellular channel 4 (*Clic4*)	1	**Yes**
Hes related family bHLH transcription factor with YRPW motif-like (*Heyl*)	3	Not tested
Chloride intracellular channel 1 (*Clic1*)	2	Not tested
Fatty acid synthase (*Fasn*)	2	Not tested
Protein disulfide isomerase family A member 4 (*Pdia4*)	2	Not tested
Germ cell-less spermatogenesis associated 1 (*Gmcl1*)	1	Not tested
Lysine acetyltransferase 5 (*Kat5*)	1	Not tested
Actin binding LIM protein 1 (*Ablim1*)	1	Not tested
Zinc finger and BTB domain containing 44 (*Zbtb44*)	1	Not tested

Table S2 Continued.
Results from mouse kidney Y2H screen

Gene name	# of clones	Confirmed by NanoSPD?
Protein phosphatase 1 catalytic subunit alpha (*Ppp1ca*)	11	**Yes**
Chromodomain helicase DNA binding protein 4 (*Chd4*)	5	**Yes**
Protein phosphatase 1 catalytic subunit gamma (*Ppp1cc*)	3	**Yes**
Chromodomain helicase DNA binding protein 3 (*Chd3*)	2	**Yes**
Chloride intracellular channel 5 (*Clic5*)	2	**Yes**
Villin-like (*Vill*)	6	Not tested
Chloride intracellular channel 1 (*Clic1*)	2	Not tested
Germ cell-less spermatogenesis associated 1 (*Gmcl1*)	2	Not tested
Epidermal growth factor receptor pathway substrate 8 (Eps8)	2	Not tested
Hes related family bHLH transcription factor with YRPW motif-like (*Heyl*)	1	Not tested
Xeroderma pigmentosum, complementation group C (*Xpc*)	1	Not tested
Family with sequence similarity 107, member B, (*Fam107b*)	1	Not tested