## SUPPLEMENTAL MATERIALS

## PICK1 is implicated in organelle motility in an Arp2/3 complex-independent manner

Yadaiah Madasu<sup>a</sup>, Changsong Yang<sup>b</sup>, Malgorzata Boczkowska<sup>a</sup>, Kelley A. Bethoney<sup>a</sup>, Adam Zwolak<sup>a</sup>, Grzegorz Rebowski<sup>a</sup>, Tatyana Svitkina<sup>b</sup>, and Roberto Dominguez<sup>a</sup>

<sup>a</sup> Department of Physiology, Perelman School of Medicine, and <sup>b</sup> Department of Biology, University of Pennsylvania, Philadelphia, PA 19104\*

Address correspondence to: Roberto Dominguez (droberto@mail.med.upenn.edu)

H1	PDZ	H2				BAR				ACT	
1 10 15		108 113 128	147	198	203	275	2 289		355 360 369	385	415
1 10 15		100 113 120	147	190 2	203	212	2 205		333 300 309	303	415
	1 10 15			0 V 0 0 0 1 V 1 1		L'BOTHLOD		,81 83 88	O FUMO FUE		105
Human Rat	MFADLDYDIEEDKLGI	PTVPGKVTLQKDAQNL	IGISIGGGA	QYCPCLYIN	/QVFDNTPA /QVFDNTPA	ALDGTVAAGD	EITGVNGRSIK	GKTKVEVAKMI	QEVKGEVT	IHYNKLQ	105
Mouse	MFADLDYDIEEDKLGI	PTVPGKVTLQKDAQNL	IGISIGGGA	QYCPCLYI	QVEDNTPA	ALDGTVAAGD	EITGVNGKSIK	GKTKVEVAKMI	QEVKGEVT	HYNKLQ	105
Orangutan	MFADLDYDIEEDKLGI	PTVPGKVTLQKDAQNL	IGISIGGGA	QYCPCLYIN	QVFDNTPA	ALDGTVAAGD	EITGVNGRSIK	GKTKVEVAKMI	QEVKGEVT	IHYNKLQ	105
Macaque Chimpanzee	MFADLDYDIEEDKLGI MFADLDYDIEEDKLGI	PTVPGKVTLQKDAQNL PTVPGKVTLQKDAQNL	G   S   GGG A   G   S   GGG A	QYCPCLYIN	/QVFDNTPA /QVFDNTPA	ALDGTVAAGD	E I TG VNGR S I K E I TG VNGR S I K	GKTKVEVAKMI GKTKVEVAKMI	QEVKGEVTI	I H Y N K L Q I H Y N K L Q	105 105
Cat	MFADLDYDIEEDKLGI	PTVPGKVTLQKDAQNL	IGISIGGGA	QYCPCLYIN		ALDGTVAAGD	EITGVNGRSIK	GKTKVEVAKMI	QEVKGEVT	HYNKLQ	105
Chicken	MFADLDYDIEEDKLGI	PTVPGTVTLKKDSQNL	IGISIGGGA	QYCPCLYIN	QVFDNTPA	ALDGTVAAGD	EITGVNGKSVK	GKTKVEVAKMI	QMVKGEVT	IHYNKLQ	105
Dog Guinea pig	MFADLDYDIEEDKLGI MFADLDYDIEEDKLGI	PTVPGKVTLQKDAQNL PTVPGKVTLQKDAQNL	G   S   GGG A   G   S   GGG A	QYC PCLYIN QYC PCLYIN	/QVFDNTPA /QVFDNTPA	ALDGTVAAGD	E I TG VNGR S I K E I TG VNG K S I K	GKTKVEVAKMI GKTKVEVAKMI	QEVKGEVTI	I HYNKLQ I HYNKLQ	105 105
Zebrafish	METDMDYEL EEDKLGI	PTVPGTVTL KKDSQNL	IGISIGGGA	QFCPCLYI	QVEDNTAA	ALDGTL AAGD	EITGVNGKPVK	GKTKVEVAKMI	QAVQGEVVI		105
Drosophila	N	ITVSTNAVVI TKDQSNL	IGISIGGGA	PMCPCLYI	QUEDNTEA /QIEDGTEA	AREGSLQSGD	ELLAVNSVSVK	GKTKVEVAKMI	QTATDEVV	I H YN KL Q	90
	108 113 119 120	122 124 128	141	47 151	///////////////////////////////////////	171			198 203		1
Human	ADPKQGMSLDIVLKKV	KHRLVENMSSGTADAL	GLSRAILCN		ELERTAELY	KGMTEHTKNL	LRAFYELSQTH	RAFGDVFSVIC	VREPOPAAS	SEAFVKF	210
Rat	ADPKQGMSLDIVLKKV	KHRLVENMSSGTADAL	GLSRAILCN	IDGLVKRLEE	ELERTAELY	KGMT EHT KNL	LRAFYEL SQTH	RAFGDVFSVIC	VREPQPAAS	SEAFVKE	210 210
Bovine	ADPKQGMSLDIVLKKV	KHRLVENMSSGTADAL	GLSRAILCN	IDGLVKRLEE	LERTAELY	KGMTEHTKNL	LRAFYELSQTH	RAFGDVFSVIC	VREPQPAAS	SEAFVKF	210
Orangutan Macaque	ADPKQGMSLDIVLKKV ADPKQGMSLDIVLKKV	KHRLVENMSSGTADAL	GL SRATLCN GL SRATLCN	IDGLVKRLEE IDGLVKRLEE	ELERTAELY ELERTAELY	KGMT EHT KNL	LRAFYEL SQTH LR <mark>V</mark> FYEL SQTH	RAFGDVFSVIC	SVREPQPAAS SVREPQPAAS	SEAFVKF	210 210
Chimpanzee		KHRLVENMSSGTADAL	GLSRAILCN		ELERTAELY	KGMT EHT KNL	LRAFYEL SQTH	RAFGDVFSVIC	VREPQPAAS	SEAFVKE	210 210
Horse	ADPKQGMSLDIVLKKV	KHRLVENMSSGTADAL	GLSRAILCN	IDGLVKRLEE	LERTAELY	KGMTEHTKNL	LRAFYELSQTH	RAFGDVFSVIC	VREPQPAAS	SEAFVKF	210
Chicken Dog	ADPKQGKSLDIVLKKV ADPKQGMSLDIVLKKV	KHRLVENMSSGTADAL KHRLVENMSSGTADAL	GL SRAILCN GL SRAILCN	IDGLVKRLEE IDGLVKRLEE	ELERTAELY ELERTAELY	KGLTEHTKSL KGMTEHTKNL	LRAFFELSQTH LRAFYELSQTH	RAFGDVFSVIO RAFGDVFSVIO	SVREPQPAAS SVREPQPAAS	SEAFVKF SEAFVKF	210 210
Guinea pig		KHRLVENMSSGTADAL	GLSRAILCN		ELERTAELY	KGMT EHT KNLI	L AFYEL SQTH	RAFGDVFSVIC		SEAFVKE	210
Frog	ADPKQGKSLDIVLKKV	KHRLVENLSSGTADAL	GLSRAILCN	IDGLVKRLEE	LEKTGEFY	RGMMEHTKRL	LRSFFELSQTH	RAFGDVFSVIC	VREPQPAAS	SEAFVKF	203
Drosophila	ADPEQGKILDIILKKL	KHK IVDNL SSNTADTL	GL S <mark>R</mark> ATLUN			KGL VEHARRM		KSFGDCFIQIS	VHEPQQRAS	SEAFRIF	195
							2				1
			251	,252	266	272	289		307	-	
Human Rat	ADAHRSIEKFGIRLLK ADAHRSIEKFGIRLLK	T I KPML TDLNTYLNKA	I PDTRLTIK I PDTRLTIK	KYLDVKFEN KYLDVKFEN	YLSYCLKVK YLSYCLKVK	EMDDEEYSCI	ALGEPL YR VST ALGEPL YR VST	GNYEYRLILRO GNYEYRLILRO	RQEARARES	SQMR KD V SQMR KD V	315 315
Mouse	ADAHRSIEKEGIRLLK	TIKPML TOLNTYLNKA		KYLDVKFEN	YLSYCLKVK	EMDDEEYSCI	ALGEPL YRVST	GNYEYRLILRO	RQEARARES		315
Orangutan	ADAHRSIEKFGIRLLK	TIKPMLTDLNTYLNKA		KYLDVKFE	YLSYCLKVK	EMDDEEYSCI	ALGEPLYRVST	GNYEYRLILRO	RQEARARFS	SQMR KD V	315
Macaque Chimpanzee	ADAHRSIEKFGIRLLK ADAHRSIEKFGIRLLK	T I KPML TDLNTYLNKA	I PDTRLTIK I PDTRLTIK	KYLDVKFEN Kyldvkfen	YLSYCLKVK YLSYCLKVK	EMDDEEYSCI	ALGEPL YR VST ALGEPL YR VST	GNYEYRLILRO GNYEYRLILRO	RQEARARES	SQMR KD V SQMR KD V	315 315
Cat	ADAHRSIEKFGIRLLK	TIKPML TOLNTYLNKA		KYLDVKFEY	YLSYCLKVK	EMDDEEYSCI	ALGEPL YRVST	GNYEYRLILRO	RQEARARES		315
Chicken	ADAHRNIEKFGIHLLK	TIKPMLTDLNTYLNKA		KYLDVKFE	YLSYCLKVK	EMDDEEYSCI	ALGEPLYRVST	GNYEYRLILRO	RQEARTRFA	AK MR KD V	315
Dog Guinea pig	ADAHRSIEKFGIRLLK ADAHRSIEKSGIQLLK	T I KPML TDLNTYLNKA	I PDTRLTIK I PDTRLTIK	KYLDVKFEY	YLSYCLKVK YLSYCLKVK	EMDDEEYSCI	ALGEPL YR VST ALGEPL YR VST	GNYEYRLILRO GNYEYRLILRO	RQEARARES	SQMR KD V SQMR KD V	315 315
Zebrafish	AEAHRNMEKEGIQLLK	TIKPMLHDLNTYLHKA		KYLDVKFEY	YLSYCLKVK	EMDDEEYSCI	ALGDPL YRVST	GNYEYRL VLRC			315
Drosophila	GEFHRTLEKDGLGIIK	QIKPVLADLGTYLNKA		RYADAKET	LSYCLKVK	EMDDEEHGFA	ALQEPLYRVET	GNYEYRLILRO	RQDARSKF	AKLRTDV	300
			35	5 360 36	64 369 3	73	385	395		415	
Human	LEKMELLDQKHVQDIV	FQLQRLVSTMSKYYND	CYAVLRD - A	DVFPIEVDL	AHTTLAYG	LNQEEFTDG-	- EEEEEEDTA	AGEPSRDTRGA	AGPLDKGG	SWCDS	415
Rat		FQLQRFVSTMSKYYND	CYAVLRD - A		AHTTLAYG	PNQGGF TDGE	- DEEEEEDGA	AREVSKDARGA	ATGPTDKGGS	SWCDS	416 416
Bovine	LEKMELLDQEHVQDIV	LQLQRFVSTMSKYYND	CYSVLRD - A	DVFPIEVDL	AHTTLAYG	LSQDEFTDGE	DEEDEDEEDTA	AGEPPRDSRG	AGPLDKGG	SWCNS	417
Orangutan Macaque		FQLQRLVSTMSKYYND	CYAVERD - A		AHTTLAYG	LNQEEFTDG-	- EEEEEEDTA	AGEPSRDTRGA	AGPLDKGGS	SWCDS	415
Chimpanzee		FQLQRLVSTMSKYYND	CYAVLRD - A		AHTTLAYG	LNQEEF TDGE		AGEPSRD TRGA		SWCDS	416 417
Horse	LEKMELLDQKHVQDIV	FQLQRFVSTMSKYYND	CYAVLRD - A	DVFPIEVDL	AHTTLAYG	LNQDEFTDGE	DEEDEDDEDTA	PGEPSRDAQG	AGPLDKGG	SWCDS	417
Chicken Dog	LEKIELLDQKHVQDIV	FQLQRFVSTMSKYYDD	CYAVLRD - A		ARTTLNYG	QKDT - YTDG - LSQDEFTDGEI	- AEEEEGGGSE DEEDEDDEDTA	RESSGKEDANC TG <mark>E</mark> PSRDAR <mark>G</mark> A	AGPLDKGG	SWCDS	408 417
Guinea pig	LEKHELLDQKHVQDIV	FQLQRFVFTMSKYYND	CYAVLES - A		AHTTLAYG	PSQGEFTDG -	- EDEDEEEGQA	AKEAAQNTRGA		SWCDS	415
Frog	LEKIELLDQKHVHDIV	FOLORFVSAMSKYNDQ			AKTTLAYG	QGDT - FTDG	DEEEEEKAEKR	NEE ENGE	KLIDDA -		398

**Figure S1. Alignment of PICK1 sequences from different organisms**. A diagram above the sequences indicates the boundaries of domains and helices of the BAR domain (according to Figure 2A in main text). Highlighted inside red boxes are positively charged amino acids that the atomic model of PICK1 suggests could be implicated in membrane interactions. Sequence conservation decreases from dark to light blue shading. The UniProt accession codes of the sequences are: Human (Q9NRD5), Rat (Q9EP80), Mouse (Q80VC8), Bovine (Q2T9M1), Orangutan (Q5REH1), Macaque (Q4R7Q5), Chimpanzee (H2QLN1), Cat (M3VZ64), Horse (F7DB33), Chicken (E1BQ62), Dog (E2RPE0), Guinea pig (H0VHQ6), Zebrafish (F1Q8Q4), Frog (Q28BS9) and Drosophila (Q86PF5). Figure related to Figure 2A in main text.



**Figure S2. Comparison of the atomic model of PICK1 with other BAR domain proteins**. (**A**) Structure of the BAR domain of arfaptin (Tarricone *et al.*, 2001), which served as a model for the BAR domain of PICK1, showing both a ribbon diagram and an electrostatic surface representation (red: negatively charged, blue: positively charged). (**B**). Model of PICK1 (according to Figure 2 in main text). (**C**) Structure of the BAR-PH domain of APPL1 (Zhu *et al.*, 2007). (**D**) Structure of the PX-BAR domain of sorting nexin 9 (Pylypenko *et al.*, 2007). Indicated above each structure are the references and accession codes of related structures in the Protein Data Bank (PDB). Note that the curvature of the BAR domain of APPL1 is similar to that of arfaptin and PICK1, whereas the curvature of the BAR domain of sorting nexin 9 is significantly less pronounced. Note also that the BAR domains of APPL1 and sorting nexin 9 are tightly associated with the PH and PX domains, respectively, through interactions that also involve accessory sequences N- and C-terminal to these domains. This provides the structural bases for association of the BAR domain with a secondary membrane-binding module, such that membrane interactions are the sum of weak interactions of the two folds, further enhanced by dimerization. This mechanism is referred to as "coincidence detection" (Moravcevic *et al.*, 2012). The model of PICK1 predicts a similar arrangement of the BAR and PDZ domains. Figure related to Figure 2 in main text.



Figure S3. Colocalization of PICK1 constructs with the *trans*-Golgi network marker TGN38.

From left to right, confocal fluorescence microscopy analysis of HeLa cells coexpressing constructs mCherry-PICK1<sub>129-415</sub>, mCherry-PICK1<sub>1-375</sub>, and mCherry-PICK1<sub>129-375</sub> (red, top row) with CFP-TGN38 (green, second row). The third row shows an overlay of the first two rows (see also Movie S5). Fourth row - zooms of the regions boxed in the third row.



**Figure S4. PICK1 binds F-actin** *in vitro*. SDS–PAGE analysis of the pellet (P) and supernatant (S) fractions of high-speed sedimentations performed at a fixed PICK1 concentration (5  $\mu$ M) and varying F-actin concentrations (0–25  $\mu$ M). The graph on the right shows the fraction of F-actin-bound PICK1 as a function of PICK1 concentration, determined from densitometric analysis of the gels. The solid line represents the global fit of the data from three independent experiments, resulting in a K<sub>D</sub> estimate of ~2.0  $\mu$ M. Yet, it must be noted that the affinity measurement from this experiment is only approximate, because the actual concentration of actin filaments vs. monomers is not precisely determined and the titration did not reach saturation. Figure related to Figure 4A in main text.



2 µM actin (6% pyrene-labeled), 20 nM Arp2/3 complex, 100 nM WCA

**Figure S5. Lack of inhibition of Arp2/3 complex polymerization by MBP-PICK1 or ACT. (A** and **B**) Time course of the fluorescence increase upon polymerization of 2 µM actin (6% pyrene-labeled) alone (black line) and with addition of the indicated proteins at the indicated protein concentrations. Experimental conditions are given on top, and each curve is color-coded. Each measurement was performed three times and one representative curve is shown. Figure related to Figure 5 in main text.

**Movie S1. 360° rotation of the MBP-PICK1 model inside the SAXS envelope**. The PICK1 domains are color-coded according to Figure 2A, and MBP is shown in green. Movie related to Figure 2B.

**Movie S2. 360° rotation of the atomic model of PICK1**. The PICK1 domains are color-coded according to Figure 2A. The side chains of positively-charged amino acids predicted to participate in membrane binding are shown. Movie related to Figure 2C.

**Movie S3. 360° rotation of an electrostatic surface representation of the PICK1 model.** Red: negatively charged, blue: positively charged. Movie related to Figure 2E.

**Movie S4. HeLa cells expressing GFP-PICK1 constructs**. From left to right the movies show cells expressing constructs GFP-PICK1, GFP-PICK1<sub>129-415</sub>, GFP-PICK1<sub>1-375</sub>, and GFP-PICK1<sub>129-375</sub> (as indicated). Each movie consists of 20 frames, taken at 3 s intervals, for a total of 60 s, and played at 10x their actual speeds. Movies related to Figure 3B.

**Movie S5. HeLa cells coexpressing PICK1 constructs and the** *trans*-Golgi marker TGN38. From left to right the movies show cells coexpressing constructs mCherry-PICK1<sub>129-415</sub>, mCherry-PICK1<sub>1-375</sub>, and mCherry-PICK1<sub>129-375</sub> (red) with CFP-TGN38 (green). Each movie consists of 20 frames, taken at 2 s intervals, for a total of 40 s, and played at 5.7x their actual speeds. Movies related to Supplemental Figure S3.

**Movie S6. HeLa cells coexpressing GFP-PICK1**<sub>129-415</sub> (green) and RFP-actin (red). The movie consists of 20 frames, taken at 3 s intervals, for a total of 60 s, and is played at 10x its actual speed. Movie related to Figure 4B.

**Movie S7. B16F1 cells expressing GFP-PICK1**<sub>129-415</sub> with or without treatment with latrunculin **B**. Each movie consists of 15 frames, taken at 4 s intervals, for a total of 60 s, and played at 10x their actual speeds. Movies related to Figure 4C.

**Movie S8. HeLa cells expressing GFP-PICK1**<sub>129-415</sub> with or without treatment with CK-666. Each movie consists of 20 frames, taken at 3 s intervals, for a total of 60 s, and played at 10x their actual speeds. Control cells were treated with DMSO. Movies related to Figure 6.

## SUPPLEMENTAL REFERENCES

- Moravcevic, K., Oxley, C.L., and Lemmon, M.A. (2012). Conditional peripheral membrane proteins: facing up to limited specificity. Structure *20*, 15-27.
- Pylypenko, O., Lundmark, R., Rasmuson, E., Carlsson, S.R., and Rak, A. (2007). The PX-BAR membrane-remodeling unit of sorting nexin 9. Embo J *26*, 4788-4800.
- Tarricone, C., Xiao, B., Justin, N., Walker, P.A., Rittinger, K., Gamblin, S.J., and Smerdon, S.J. (2001). The structural basis of Arfaptin-mediated cross-talk between Rac and Arf signalling pathways. Nature 411, 215-219.
- Zhu, G., Chen, J., Liu, J., Brunzelle, J.S., Huang, B., Wakeham, N., Terzyan, S., Li, X., Rao, Z., Li, G., and Zhang, X.C. (2007). Structure of the APPL1 BAR-PH domain and characterization of its interaction with Rab5. Embo J 26, 3484-3493.