Proximity interactions of the ubiquitin ligase Mind bomb 1 reveal a

role in regulation of epithelial polarity complex proteins

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Supplementary Table S2: GO Enrichment Analysis

Annotation Cluster 1	Enrichment Sc	ore: 7.757390046319508											
Category	Term		Count %	6	PValue Gen	ies	List Total	Pop Hits P	op Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_BP_DIRECT	GO:000086	G2/M transition of mitotic cell cycle	15	9.62	7.27982E-12 933	23, 510	140	137	16792	13.13	5.47E-09	2.74E-09	1.11E-08
GOTERM_BP_DIRECT	GO:0051297	centrosome organization	9	5.77	2.09838E-10 933	23, 117	140	33	16792	32.71	1.58E-07	5.26E-08	3.19E-07
GOTERM_CC_DIRECT	GO:0070652	HAUS complex	6	3.85	1.75168E-09 933	23, 794	148	8	18224	92.35	4.10E-07	1.02E-07	2.25E-06
GOTERM_BP_DIRECT	GO:0051225	spindle assembly	7	4.49	7.40592E-08 933	23, 794	140	27	16792	31.10	5.57E-05	1.39E-05	1.13E-04
GOTERM_BP_DIRECT	GO:0007067	mitotic nuclear division	14	8.97	1.45417E-07 933	23, 794	140	248	16792	6.77	1.09E-04	2.19E-05	2.21E-04
GOTERM_BP_DIRECT	GO:0051301	cell division	16	10.26	2.12656E-07 933	23, 784	140	350	16792	5.48	1.60E-04	2.67E-05	3.24E-04
GOTERM_CC_DIRECT	GO:0005874	microtubule	15	9.62	2.30558E-07 933	23, 784	148	311	18224	5.94	5.39E-05	8.99E-06	2.96E-04
GOTERM_CC_DIRECT	GO:0005819	spindle	9	5.77	6.17682E-06 7984	48, 794	148	121	18224	9.16	0.00144434	1.81E-04	0.00794045
Annotation Cluster 2	Enrichment Sc	ore: 4.376357251331086											
Category	Term		Count %	, D	PValue Gen	ies	List Total	Pop Hits P	op Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_BP_DIRECT	GO:0042059	negative regulation of epidermal growth factor rece	11	7.05	2.30333E-13 161,	, 163, 5	140	36	16792	36.65	1.73E-10	1.73E-10	3.51E-10
GOTERM_CC_DIRECT	GO:0030122	AP-2 adaptor complex	7	4.49	4.07201E-10 161,	, 163, 9	148	13	18224	66.30	9.53E-08	3.18E-08	5.23E-07
GOTERM_BP_DIRECT	GO:0072583	clathrin-mediated endocytosis	6	3.85	5.07274E-07 161,	, 163, 1	140	20	16792	35.98	3.81E-04	5.45E-05	7.72E-04
GOTERM_MF_DIRECT	GO:0035615	clathrin adaptor activity	5	3.21	8.15198E-07 161,	, 163, 1	137	10	16881	61.61	1.73E-04	8.64E-05	0.00103151
GOTERM_CC_DIRECT	GO:0036020	endolysosome membrane	5	3.21	1.91297E-06 161,	, 163, 1	148	12	18224	51.31	4.48E-04	6.39E-05	0.00245922
GOTERM_BP_DIRECT	GO:0006886	intracellular protein transport	11	7.05	2.71801E-05 161,	, 1020,	140	236	16792	5.59	0.02023223	0.00204188	0.0413743
GOTERM_BP_DIRECT	GO:0048013	ephrin receptor signaling pathway	7	4.49	7.88401E-05 161,	, 163, 6	140	86	16792	9.76	0.05756666	0.00492865	0.11996853
GOTERM_BP_DIRECT	GO:0050690	regulation of defense response to virus by virus	5	3.21	7.88908E-05 161,	, 163, 1	140	28	16792	21.42	0.05760258	0.00455331	0.12004562
GOTERM_BP_DIRECT	GO:0048268	clathrin coat assembly	4	2.56	0.000149392 163,	, 11554	140	13	16792	36.91	0.10626989	0.00699737	0.22721172
GOTERM_CC_DIRECT	GO:0030666	endocytic vesicle membrane	6	3.85	0.000191899 161,	, 163, 1	148	66	18224	11.19	0.04391524	0.00407429	0.24641938
GOTERM_CC_DIRECT	GO:0030669	clathrin-coated endocytic vesicle membrane	5	3.21	0.000326321 161,	, 163, 1	148	41	18224	15.02	0.07352803	0.00448236	0.4186976
GOTERM_BP_DIRECT	GO:0007018	microtubule-based movement	6	3.85	0.000559468 161,	, 163, 1	140	81	16792	8.88	0.34350333	0.02445126	0.84842145
GOTERM_BP_DIRECT	GO:0060071	Wnt signaling pathway, planar cell polarity pathway	6	3.85	0.000998721 161,	, 163, 1	140	92	16792	7.82	0.5283006	0.03687365	1.50982109
GOTERM_BP_DIRECT	GO:0019886	antigen processing and presentation of exogenous	5	3.21	0.007161458 161,	, 163, 1	140	92	16792	6.52	0.99550501	0.15540645	10.3652509
GOTERM_CC_DIRECT	GO:0030131	clathrin adaptor complex	3	1.92	0.007200785 161,	, 163, 1	148	16	18224	23.09	0.81567958	0.07398723	8.8720897
GOTERM_CC_DIRECT	GO:0030117	membrane coat	3	1.92	0.018503454 161,	, 163, 1	148	26	18224	14.21	0.98735341	0.14944437	21.3453315
GOTERM_MF_DIRECT	GO:0008565	protein transporter activity	4	2.56	0.020368871 161,	, 163, 1	137	72	16881	6.85	0.98725716	0.32740834	22.9255495
GOTERM_BP_DIRECT	GO:0016192	vesicle-mediated transport	4	2.56	0.131917287 161,	, 163, 1	140	152	16792	3.16	1	0.81522884	88.3967684
Annotation Cluster 3	Enrichment Sc	ore: 2.9147289108024097											
Category	Term		Count %	b	PValue Gen	ies	List Total	Pop Hits P	op Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_CC_DIRECT	GO:0016282	eukaryotic 43S preinitiation complex	4	2.56	0.000217906 8662	2, 8661	148	15	18224	32.84	0.04971708	0.00391505	0.27977174
GOTERM_CC_DIRECT	GO:0033290	eukaryotic 48S preinitiation complex	4	2.56	0.000217906 8662	2, 8661	148	15	18224	32.84	0.04971708	0.00391505	0.27977174
GOTERM_CC_DIRECT	GO:0005852	eukaryotic translation initiation factor 3 complex	4	2.56	0.00032183 8663	2, 8661	148	17	18224	28.97	0.07255356	0.00469645	0.41294612
GOTERM_BP_DIRECT	GO:0001731	formation of translation preinitiation complex	4	2.56	0.000870674 8662	2, 8661	140	23	16792	20.86	0.48057458	0.03388787	1.31744302
GOTERM_CC_DIRECT	GO:0071541	eukaryotic translation initiation factor 3 complex, e	3	1.92	0.00132157 8663	2, 8661	148	7	18224	52.77	0.26615096	0.01535351	1.68572492
GOTERM_MF_DIRECT	GO:0003743	translation initiation factor activity	5	3.21	0.001474097 947	0, 8662	137	61	16881	10.10	0.26855923	0.06063185	1.84930867
GOTERM_BP_DIRECT	GO:0006446	regulation of translational initiation	4	2.56	0.00324534 8662	2, 8661	140	36	16792	13.33	0.91322743	0.08359999	4.82866524
GOTERM_BP_DIRECT	GO:0006413	translational initiation	6	3.85	0.005685435 947	0, 8662	140	137	16792	5.25	0.9862628	0.12917236	8.31478483
GOTERM_MF_DIRECT	GO:0031369	translation initiation factor binding	3	1.92	0.012239971 8663	2, 7286	137	21	16881	17.60	0.92653052	0.25181002	14.429874
Annotation Cluster 4	Enrichment Sc	ore: 2.2774804592397127											
Category	Term		Count %	Ď	PValue Gen	ies	List Total	Pop Hits P	op Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_CC_DIRECT	GO:0033565	ESCRT-0 complex	3	1.92	0.000192849 102	54, 802	148	3	18224	123.14	0.04412781	0.00375386	0.24763798
GOTERM_BP_DIRECT	GO:0016197	endosomal transport	5	3.21	0.002178226 102	54, 887	140	66	16792	9.09	0.80598365	0.0634876	3.26550849
GOTERM_CC_DIRECT	GO:0031901	early endosome membrane	5	3.21	0.014504528 3999	979, 20	148	116	18224	5.31	0.96725205	0.12781751	17.1245603

GOTERM_BP_DIRECT	GO:0006914	autophagy	5	3.21	0.024870216	9779, 102	5 140	133	16792	4.51	0.99999999	0.36992999	31.8488962
GOTERM_BP_DIRECT	GO:0036258	multivesicular body assembly	3	1.92	0.027045418	10254, 80	2 140	31	16792	11.61	1	0.38090344	34.1271211
Annotation Cluster 5	Enrichment Sc	ore: 2.1743126331310445											
Category	Term		Count %	D	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_BP_DIRECT	GO:0070911	global genome nucleotide-excision repair	5	3.21	0.000135044	7341, 845	1 140	32	16792	18.74	0.09657293	0.00722803	0.20541007
GOTERM_BP_DIRECT	GO:0000717	nucleotide-excision repair, DNA duplex unwinding	4	2.56	0.000761703	8451, 845	C 140	22	16792	21.81	0.4361794	0.03133302	1.15344862
GOTERM_BP_DIRECT	GO:0000715	nucleotide-excision repair, DNA damage recognitio	4	2.56	0.000870674	8451, 845	C 140	23	16792	20.86	0.48057458	0.03388787	1.31744302
GOTERM_BP_DIRECT	GO:0006294	nucleotide-excision repair, preincision complex ass	4	2.56	0.001732438	8451, 845	C 140	29	16792	16.54	0.72853528	0.05511524	2.60542093
GOTERM_BP_DIRECT	GO:0042769	DNA damage response, detection of DNA damage	4	2.56	0.00324534	8451, 845	C 140	36	16792	13.33	0.91322743	0.08359999	4.82866524
GOTERM_BP_DIRECT	GO:0006296	nucleotide-excision repair, DNA incision, 5'-to lesion	4	2.56	0.0035105	8451, 845	C 140	37	16792	12.97	0.928962	0.0871567	5.21340489
GOTERM_BP_DIRECT	GO:0033683	nucleotide-excision repair, DNA incision	4	2.56	0.003788536	8451, 845	C 140	38	16792	12.63	0.94240902	0.09076011	5.61526719
GOTERM_BP_DIRECT	GO:0006293	nucleotide-excision repair, preincision complex stal	3	1.92	0.012888035	8451, 845	C 140	21	16792	17.13	0.99994198	0.24323955	17.9218784
GOTERM_BP_DIRECT	GO:0006295	nucleotide-excision repair, DNA incision, 3'-to lesion	r 3	1.92	0.015360668	8451, 845	C 140	23	16792	15.64	0.9999912	0.26386252	20.9970071
GOTERM_BP_DIRECT	GO:0006283	transcription-coupled nucleotide-excision repair	4	2.56	0.02347942	8451, 845	C 140	74	16792	6.48	0.99999998	0.36024995	30.3538701
GOTERM_BP_DIRECT	GO:0042787	protein ubiquitination involved in ubiquitin-depend	l 5	3.21	0.038386209	5347, 845	1 140	153	16792	3.92	1	0.46542219	44.8961999
GOTERM_MF_DIRECT	GO:0061630	ubiquitin protein ligase activity	4	2.56	0.191583872	8315, 845	1 137	187	16881	2.64	1	0.84720509	93.2195215
GOTERM_BP_DIRECT	GO:0043161	proteasome-mediated ubiquitin-dependent proteir	4	2.56	0.236724205	8451, 997	8 140	203	16792	2.36	1	0.93336745	98.3639403
Annotation Cluster 6	Enrichment Sc	ore: 1.953777794242805											
Category	Term		Count %	>	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_BP_DIRECT	GO:0070423	nucleotide-binding oligomerization domain contain	5	3.21	4.9689E-05	23118, 10	4 140	25	16792	23.99	0.03667749	0.00339124	0.07562583
GOTERM_CC_DIRECT	GO:0010008	endosome membrane	8	5.13	0.000766167	9779, 231	1 148	185	18224	5.32	0.1641882	0.00991451	0.9805006
GOTERM_BP_DIRECT	GO:0007179	transforming growth factor beta receptor signaling	6	3.85	0.000998721	9958, 473	8 140	92	16792	7.82	0.5283006	0.03687365	1.50982109
GOTERM_BP_DIRECT	GO:0007249	I-kappaB kinase/NF-kappaB signaling	5	3.21	0.001531154	23118, 10	4 140	60	16792	10.00	0.68409379	0.05102965	2.30599846
GOTERM_BP_DIRECT	GO:0002755	MyD88-dependent toll-like receptor signaling path	4	2.56	0.002525136	23118, 10	4 140	33	16792	14.54	0.85062631	0.06799647	3.77629028
GOTERM_BP_DIRECT	GO:0007254	JNK cascade	4	2.56	0.007745364	23118, 10	4 140	49	16792	9.79	0.99711199	0.16237727	11.1645115
GOTERM_BP_DIRECT	GO:0002223	stimulatory C-type lectin receptor signaling pathwa	4	2.56	0.056566796	23118, 10	4 140	105	16792	4.57	1	0.56919134	58.7929391
GOTERM_BP_DIRECT	GO:0000187	activation of MAPK activity	4	2.56	0.059197228	23118, 10	4 140	107	16792	4.48	1	0.57929277	60.5079196
GOTERM_BP_DIRECT	GO:0051092	positive regulation of NF-kappaB transcription factor	4	2.56	0.099240686	23118, 10	4 140	133	16792	3.61	1	0.7360911	79.6338885
GOTERM_BP_DIRECT	GO:0043123	positive regulation of I-kappaB kinase/NF-kappaB s	i 4	2.56	0.149241293	23118, 68	8 140	161	16792	2.98	1	0.84586046	91.4633674
GOTERM_BP_DIRECT	GO:0038095	Fc-epsilon receptor signaling pathway	4	2.56	0.183253713	23118, 10	4 140	178	16792	2.70	1	0.89689568	95.413227
GOTERM_BP_DIRECT	GO:0050852	T cell receptor signaling pathway	3	1.92	0.346576489	23118, 68	8 140	148	16792	2.43	1	0.9807568	99.8464328
Annotation Cluster 7	Enrichment Sc	ore: 1.4814722409035246											
Category	Term		Count %		PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_CC_DIRECT	GO:0005913	cell-cell adherens junction	8	5.13	0.015957793	117178, 2	E 148	323	18224	3.05	0.97681474	0.134786	18.6820284
GOTERM_MF_DIRECT	GO:0098641	cadherin binding involved in cell-cell adhesion	7	4.49	0.030094909	26058, 91	.3 137	290	16881	2.97	0.99846327	0.39244652	32.067342
GOTERM_BP_DIRECT	GO:0098609	cell-cell adhesion	6	3.85	0.074837179	26058, 91	3 140	271	16792	2.66	1	0.66150149	69.4040448
Annotation Cluster 8	Enrichment Sc	ore: 1.4668518040035676											
Category	Term		Count %		PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_BP_DIRECT	GO:0035735	intraciliary transport involved in cilium morphogen	£ 3	1.92	0.001390288	117178, 5	1 140	7	16792	51.40	0.6487385	0.04859953	2.09594166
GOTERM_BP_DIRECT	GO:0060271	cilium morphogenesis	4	2.56	0.104362232	117178, 5	1 140	136	16792	3.53	1	0.748777	81.3273939
GOTERM_BP_DIRECT	GO:0042384	cilium assembly	3	1.92	0.274028878	117178, 5	1 140	124	16792	2.90	1	0.95256564	99.2371036
Annotation Cluster 9	Enrichment Sc	ore: 1.0790518054724672				_		_					
Category	Term		Count %	, 	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_MF_DIRECT	GO:0004843	thiol-dependent ubiquitin-specific protease activity	4	2.56	0.026794823	9958, 735	3 137	80	16881	6.16	0.99684256	0.38111315	29.0839281

GOTERIVI_IVIF_DIRECT	GO:0036459	thiol-dependent ubiquitinyl hydrolase activity	3	1.92	0.109311533	9958, 823	IS 137	70	16881	5.28	1	0.68920768	76.8869921
GOTERM_BP_DIRECT	GO:0016579	protein deubiquitination	3	1.92	0.197755109	9958, 823	s 140	99	16792	3.63	1	0.90940911	96.5081803
Annotation Cluster 10	Enrichment Sc	ore: 0.7601816526828035											
Category	Term		Count %		PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichm	Bonferroni	Benjamini	FDR
GOTERM_BP_DIRECT	GO:0018105	peptidyl-serine phosphorylation	4	2.56	0.08533973	1020, 534	7 140	125	16792	3.84	1	0.69175118	74.2858651
GOTERM_MF_DIRECT	GO:0004674	protein serine/threonine kinase activity	6	3.85	0.187108687	1020, 534	17 137	376	16881	1.97	1	0.8518389	92.728948
GOTERM_MF_DIRECT	GO:0004672	protein kinase activity	5	3.21	0.32825385	1020, 534	137	359	16881	1.72	1	0.94544804	99.3490567
Annotation Cluster 11	Enrichment Sc	ore: 0 735256209049127											
Category	Term		Count %		PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichm	Bonferroni	Benjamini	FDR
Category GOTERM_CC_DIRECT	Term GO:0005667	transcription factor complex	Count % 6	3.85	PValue 0.020210022	Genes 7027, 187	List Total 76 148	Pop Hits 193	Pop Total 18224	Fold Enrichm 3.83	Bonferroni 0.99158372	Benjamini 0.15686493	FDR 23.0854708
Category GOTERM_CC_DIRECT GOTERM_MF_DIRECT	Term GO:0005667 GO:0046983	transcription factor complex protein dimerization activity	Count % 6 5	3.85 3.21	PValue 0.020210022 0.033174901	Genes 7027, 187 23269, 18	List Total 76 148 37 137	Pop Hits 193 150	Pop Total 18224 16881	Fold Enrichm 3.83 4.11	Bonferroni 0.99158372 0.99921701	Benjamini 0.15686493 0.37924931	FDR 23.0854708 34.7470641
Category GOTERM_CC_DIRECT GOTERM_MF_DIRECT GOTERM_MF_DIRECT	Term GO:0005667 GO:0046983 GO:0003677	transcription factor complex protein dimerization activity DNA binding	Count % 6 5 14	3.85 3.21 8.97	PValue 0.020210022 0.033174901 0.598099143	Genes 7027, 187 23269, 18 84108, 23	List Total 76 148 87 137 80 137	Pop Hits 193 150 1674	Pop Total 18224 16881 16881	Fold Enrichm 3.83 4.11 1.03	Bonferroni 0.99158372 0.99921701 1	Benjamini 0.15686493 0.37924931 0.99599971	FDR 23.0854708 34.7470641 99.9990211
Category GOTERM_CC_DIRECT GOTERM_MF_DIRECT GOTERM_MF_DIRECT GOTERM_BP_DIRECT	Term GO:0005667 GO:0046983 GO:0003677 GO:0006351	transcription factor complex protein dimerization activity DNA binding transcription, DNA-templated	Count % 6 5 14 16	3.85 3.21 8.97 10.26	PValue 0.020210022 0.033174901 0.598099143 0.662541159	Genes 7027, 187 23269, 18 84108, 23 84108, 10	List Total 76 148 87 137 80 137 99 140	Pop Hits 193 150 1674 1955	Pop Total 18224 16881 16881 16792	Fold Enrichm F 3.83 4.11 1.03 0.98	Bonferroni 0.99158372 0.99921701 1 1	Benjamini 0.15686493 0.37924931 0.99599971 0.99986077	FDR 23.0854708 34.7470641 99.9990211 99.9999934



Figure S1. Expression of Flag-EPB41L5/IRES/GFP and T7-MIB1 in Hela cells. Representative immunoblots showing the relative expression of transfected proteins. Equal cell numbers from the cell preparations depicted in Figure 5 were lysed in 2xLaemmli sample buffer and immunoblotted for the transfected proteins as indicated.

В pFlag EPB41L5 No. of Peptides Bait Common Name of Prev Protein GI eln1 eln2 eln1 eln2 EPB41L5 a. Mindbomb (MIB1) 12 30348954 b. NaK Beta 3 subunit 1 15080119 13 c. Zona Occludens 1 (ZO-1) 62945799 e d. Zona Occludens 2 (ZO-2) 19 13634076 e. Crumbs1 (CRB1) 2 6912322 d f. Pinin 18 33356174 f

A

b

Figure S2. Identification of EPB41L5 interacting proteins. (A) Representative image of a colloidal Coomassie stained gel used in the identification of EPB41L5 binding partners by LC MS/MS. HEK293T cell lines stably expressing either pFLAG or pFLAG-EPB41L5 were lysed and immunoprecipitated with anti-FLAG M2 affinity gel. Proteins were eluted from the matrix using a 3X FLAG peptide and resolved by NuPage gel electrophoresis. Bands corresponding to proteins unique to the EPB41L5 elutions were excised, processed and subjected to LC MS/MS. (B) A representative summary of the prevalent proteins identified by the mass spectrometer is listed and denoted on the gel. Eln, elution.

Mass Spectrometry Protein Sample Preparation: HEK293T stable cell lines over expressing FLAG-EPB41L5 were used in mass spectrometry experiments to identify novel EPB41L5 binding partners. Sample preparation including cell lysis, immunoprecipitation and mass spectrometry (LC-MS/MS) was performed as previously described (Chen and Gingras, 2007). Briefly, 18 150 mm tissue culture plates of 90% confluent cells expressing either pFLAG empty vector or pFLAG-EPB41L5 were lysed in MS lysis buffer [50 mM HEPES-KOH pH 7.5, 150 mM NaCl, 1mM EGTA, 10% glycerol, 0.5% Triton-X100, 100 M ZnCl_a, 1 mM Na3VO4, 20 mM β-glycerolphosphate, 10 mM NaF, 1 mM DTT, 10mM Na, P₂O₂ supplemented with 1 mM PMSF, 1mM MgCl2, and 1 COMPLETE protease inhibitor tablet (Roche)], and subjected to two freeze-thaw cycles. Lysates were precleared with sepharose beads, and subjected to anti-FLAG immunoprecipitation (FLAG M2-agarose, Sigma) to isolate EPB41L5 interacting proteins. Immunoaffinity purified proteins were then eluted using 3XFLAG peptide and separated by SDS-PAGE using the NuPage system on a 4-12% gradient gel. The gel was fixed in 50% methanol/7% Acetic Acid and stained with GelCode Blue (Pierce). Gel bands were excised and The Mass Spectrometry Facility/Advanced Protein Technology Centre at The Hospital for Sick Children processed samples and performed LC-MS/MS analyses.



Figure S3. MIB1 relocalization to the plasma membrane when co-expressed with EPB41L5 requires the EPB41L5 interaction region. Polarized MDCK cells co-transfected with EGFP and HA-MIB1, EGFP-EPB41L5 and HA-MIB1, EGFP-EPB41L5 and HA-MIB1(N-Ter), EGFP-EPB41L5 and HA-MIB1(C-Ter), EGFP-EPB41L5 and HA-MIB1(Mid) where fixed and stained for HA (red). Shown are XY sections taken across the middle of the cell monolayer and XZ sections of the merge (bottom). Images were acquired at 63X magnification on a Quorum spinning disk microscope. Scale bars indicate 10 µm.

Supplementary Figure S4: Original western blots showing cropped regions used to prepare figures in the main text.

- a. Figure 2a, b and c original blots
- b. Figure 2d and 2e original blots
- c. Figure 3a and 3c original blots
- d. Figure 3d original blots
- e. Figure 3e original blots
- f. Figure 3f original blots
- g. Figure 4a 3b and 4b original blots
- h. Figure 4c original blots
- i. Figure 6b original blots
- j. Figure 6c original blots
- k. Figure 6e original blots
- l. Figure 7a
- m. Figure 7b

FIGURE 2A, 2B and 2C original blots

Fig2A Fig2B		Fig2C
StringODC14 V KIAA0753-3Flag	Flag-OFD1	Flag-OFD1
v	WT CA DOR	V WT CA D3R
245-		
135-	135-	
	# · · · · · · · · · · · · · · · · · · ·	
And the second s		
The second secon		
anti-Flag M2 1/1000		anti-Flag 10s exposure
30s exposure 30s	exposure 10s exposure	





FIGURE 2D and 2E original blots

Fig 2D







Fig 2D





FIGURE 3A and 3C original blots

Fig 3A



Fig 3A





Fig 3C



Fig 3C



Fig 3C

HA-AP2B1 <u>T7-Mib</u> v wt_ca_d3R



FIGURE 3D original blots















Figure 3f original blots



HIS6-Ub+ HA-AP2B1





FIGURE 3B, 4A and 4B original blots







Fig 4B







Coomassie

FIGURE 4C original blots



Figure 6B original blots

lgG IP αFlagM2		Lycates(5% Input)
Flag-EPB41L5 +		Flag-EPB41L5 +
Vector MIB1-FL MIB1-Nterm MIB1-Mid MIB1-Cterm MIB1-∆3R MIB1-∆RF3		AlB1-FL AlB1-Nterm AlB1-AB1-Cterm AlB1-A3R AlB1-ARF3
Blot anti-HA		Blot anti-HA
ACTAILUAS CITATO		
IBC Ib αElagM5 Vector MIB1-FL MIB1-FL MIB1-Mid MIB1-ABA MIB1-ABA MIB1-ARF3 MIB1-ARF3		Lysates(5% Input) Flag-EPB41L5 + WIB1-Vterm WIB1-Vterm WIB1-Vterm WIB1-Vterm WIB1-Vterm WIB1-Vterm WIB1-Vterm WIB1-Vterm WIB1-Vterm WIB1-Free WIB1-Vterm WIB1-Free WIB1-Vterm WIB1-Free WI
	skiwoi hoprat	
Blot anti-EPB41L5 Reblat & (Mar. Moos)	() 25/7102 dens/dramol	KYNOI LYSOLO Blot anti-EPB41L5

Figure 6C original blots





<u>Flag-</u> EPB41L5 +





Coomassie

Figure 6E original blots



1-Vector

- 2- EPB41L5 + MIB1wt
- 3- EPB41L5
- 4- EPB41L5∆PDB
- 5- EPB41L5 (1-660)
- 6- EPB41L5Nterm+FERM/FA
- 7- EPB41L5 (240-733)
- 8- EPB41L5 (333-733)
- 9- EPB41L5∆FA
- 10- EPB41L5∆FERM/FA

1 2 3 4 5 6 7 8 9 10





IP anti-Flag Blot anti-Flag

1 2 3 4 5 6 7 8 9 10



Figure 7A original blots





Lysates blot anti-EPB41L5



- 1- CRB1 + MIB1wt + EPB41L5
- 2-Vector
- 3- MIB1wt
- 4- MIB1wt + EPB41L5
- 5- MIB1CA
- 6- MIB1CA + EPB41L5
- 7- MIB1wt
- 8- MIB1wt + EPB41L5
- 9- MIB1CA
- **10-** MIB1CA + EPB41L5

Figure 7B original blots







