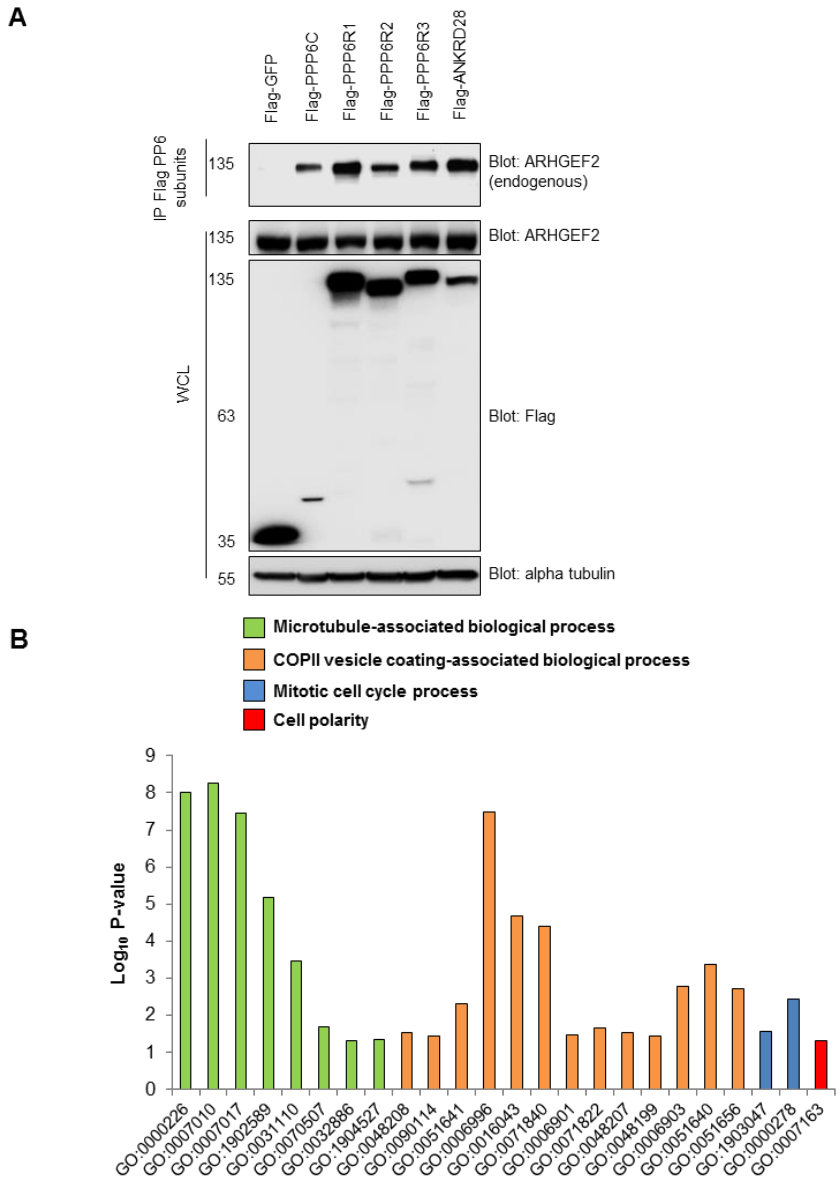
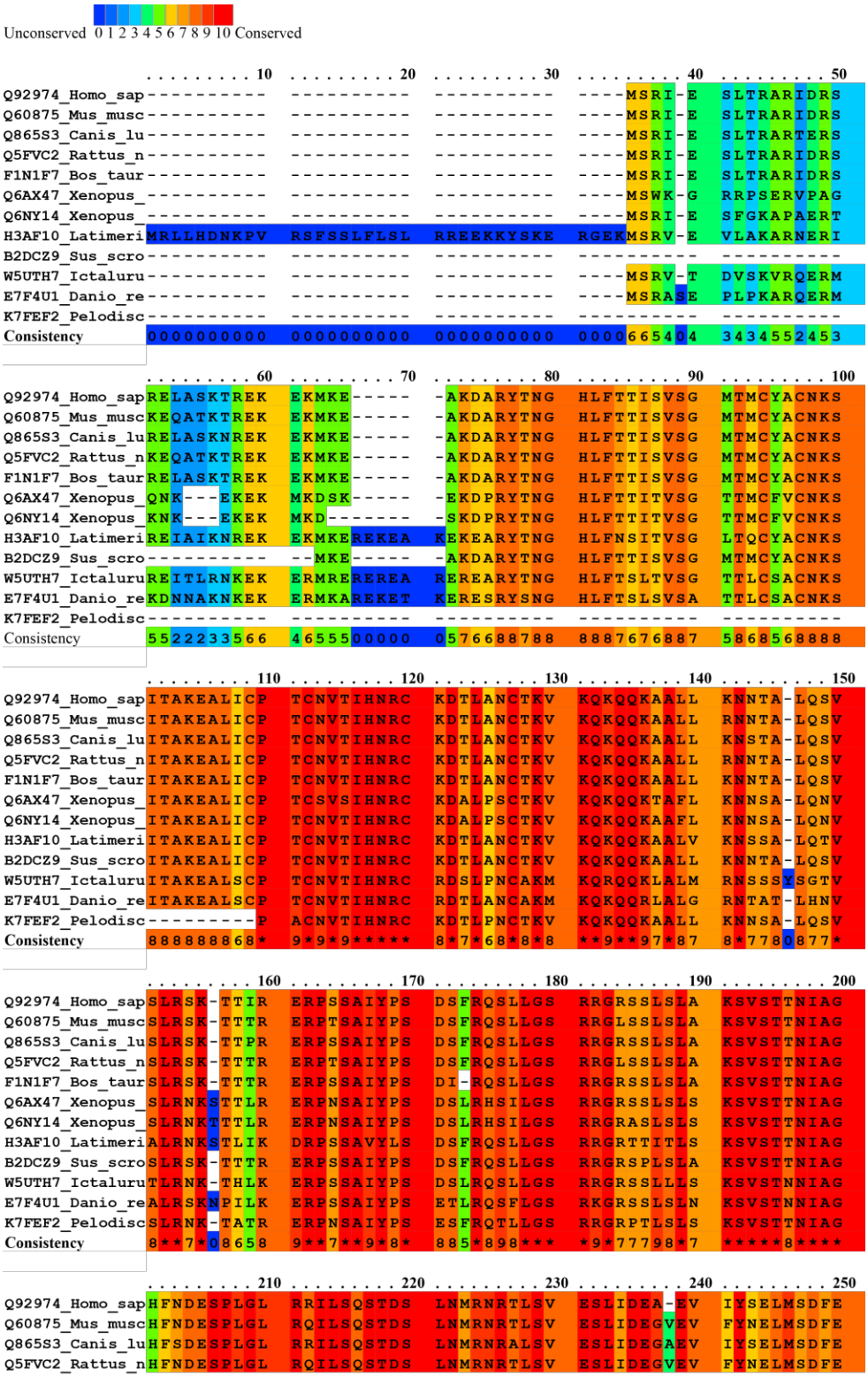


Figure S1. ARHGEF2 interaction network.

(A) Validation of a subset of ARHGEF2 interactors. Tetracycline-inducible Flag-tagged PP6 regulatory subunits were immunoprecipitated with an antibody specific for Flag covalently attached to agarose beads and protein complexes were probed for the presence of endogenous ARHGEF2. Specific antibodies for ARHGEF2 and Flag were used to detect protein abundance in cell lysates. α-tubulin was used as a loading control. Representative of two independent experiments. (B) Gene ontology (GO) enrichment analysis of the ARHGEF2 interaction network (http://www.geneontology.org/), based on biological process. See **Table S2** for more details.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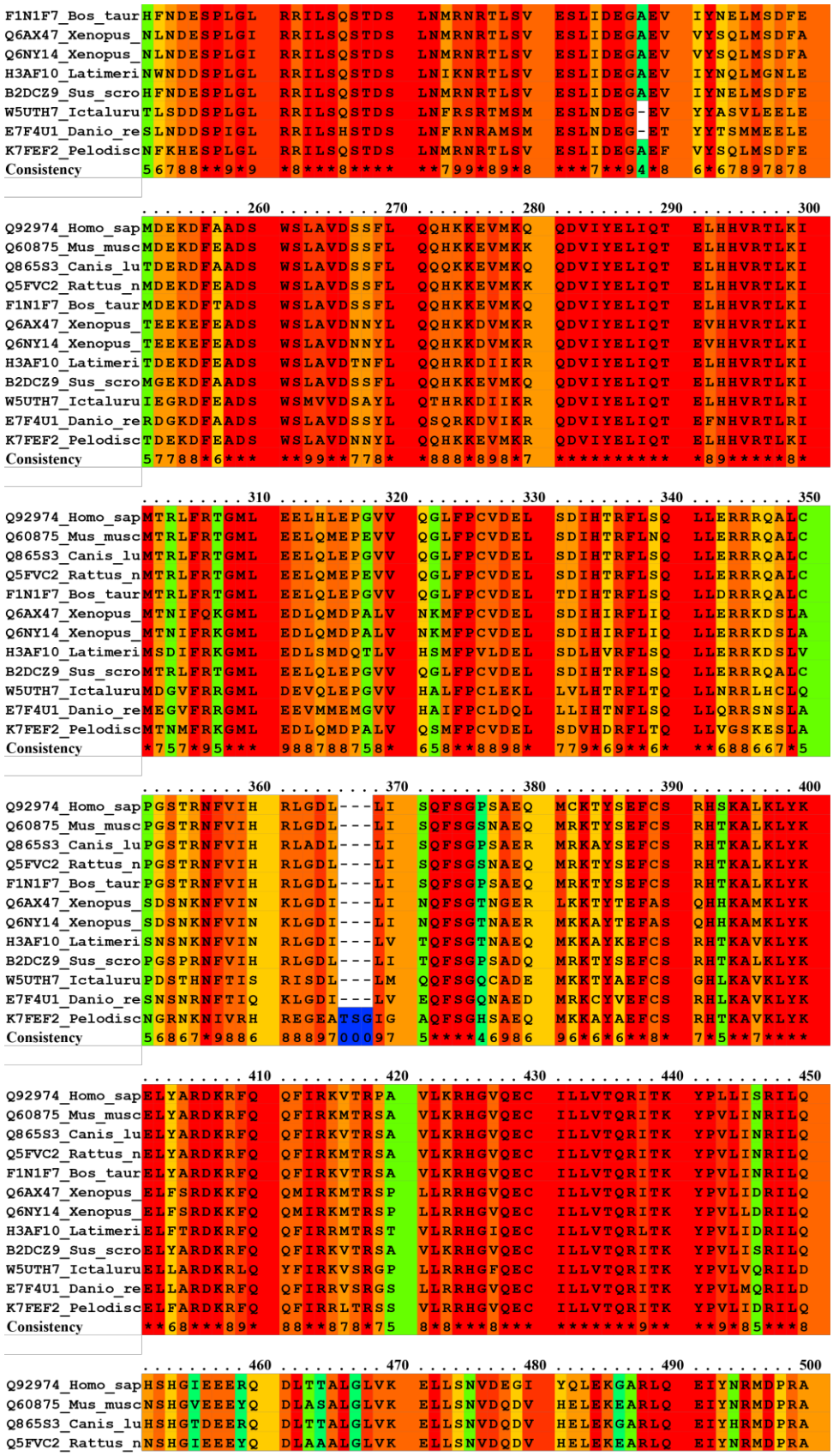
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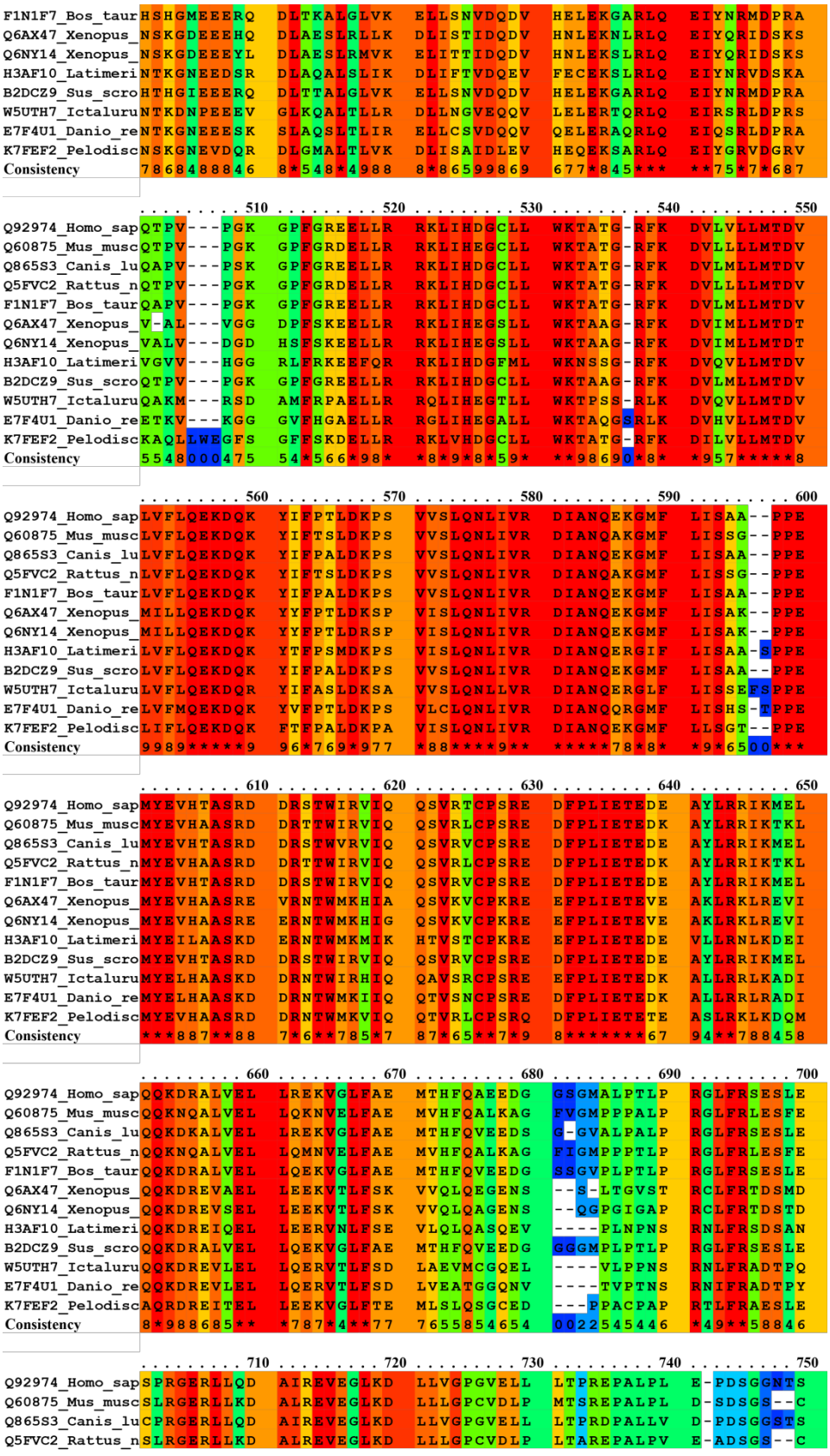
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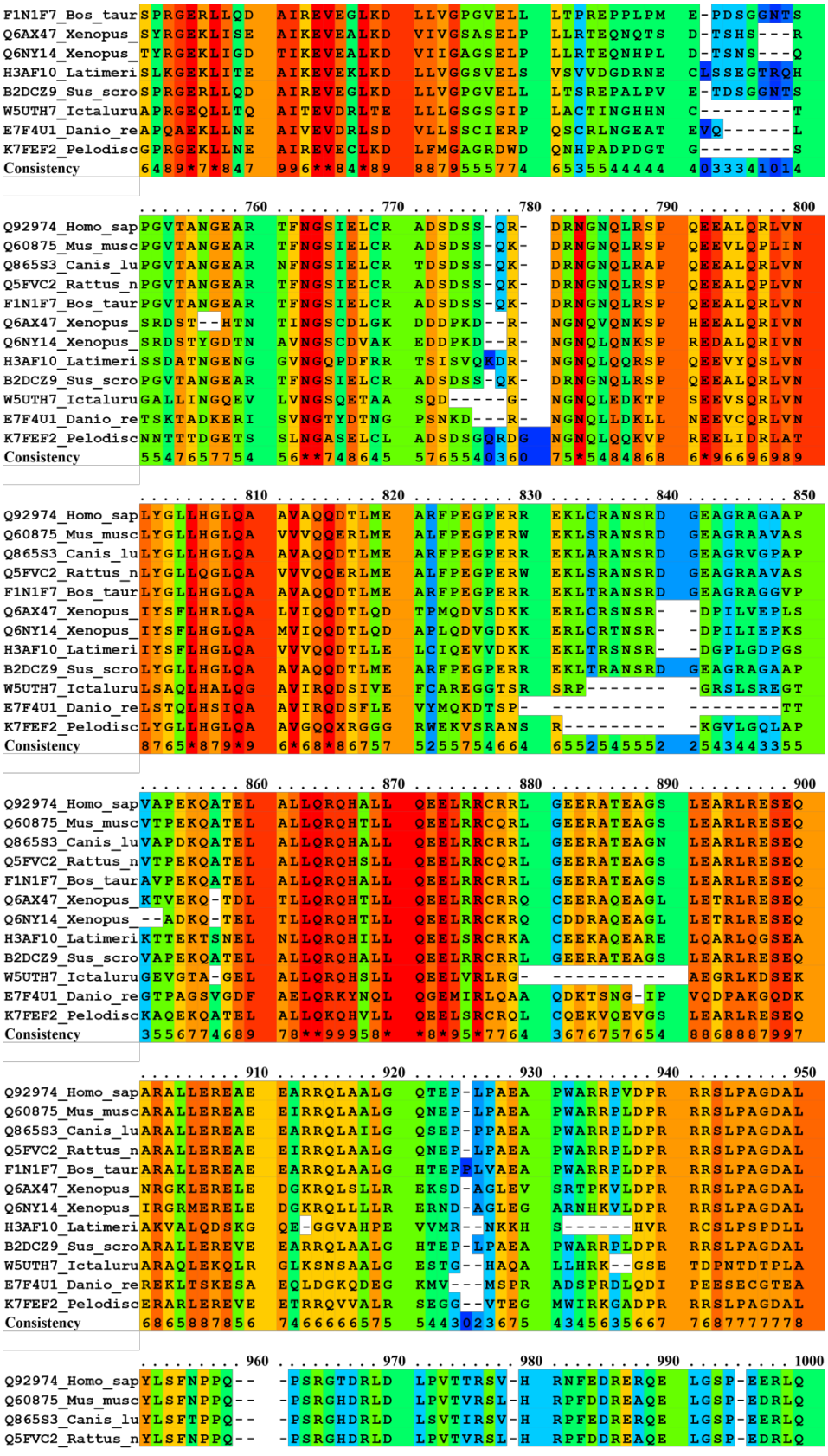
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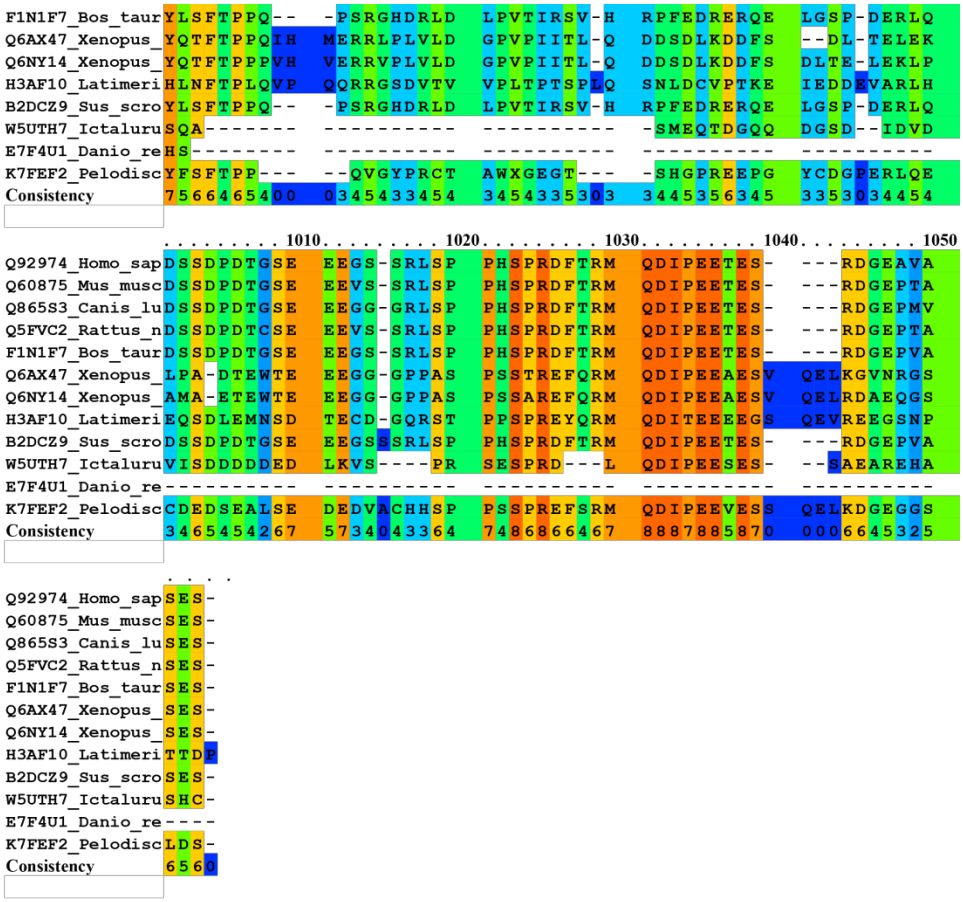

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Figure S2. Full alignment of ARHGEF2 orthologs in vertebrates.
The color code is based on the conserved residues with red or 10 being the most conserved. The conservation score is performed by PRALINE.

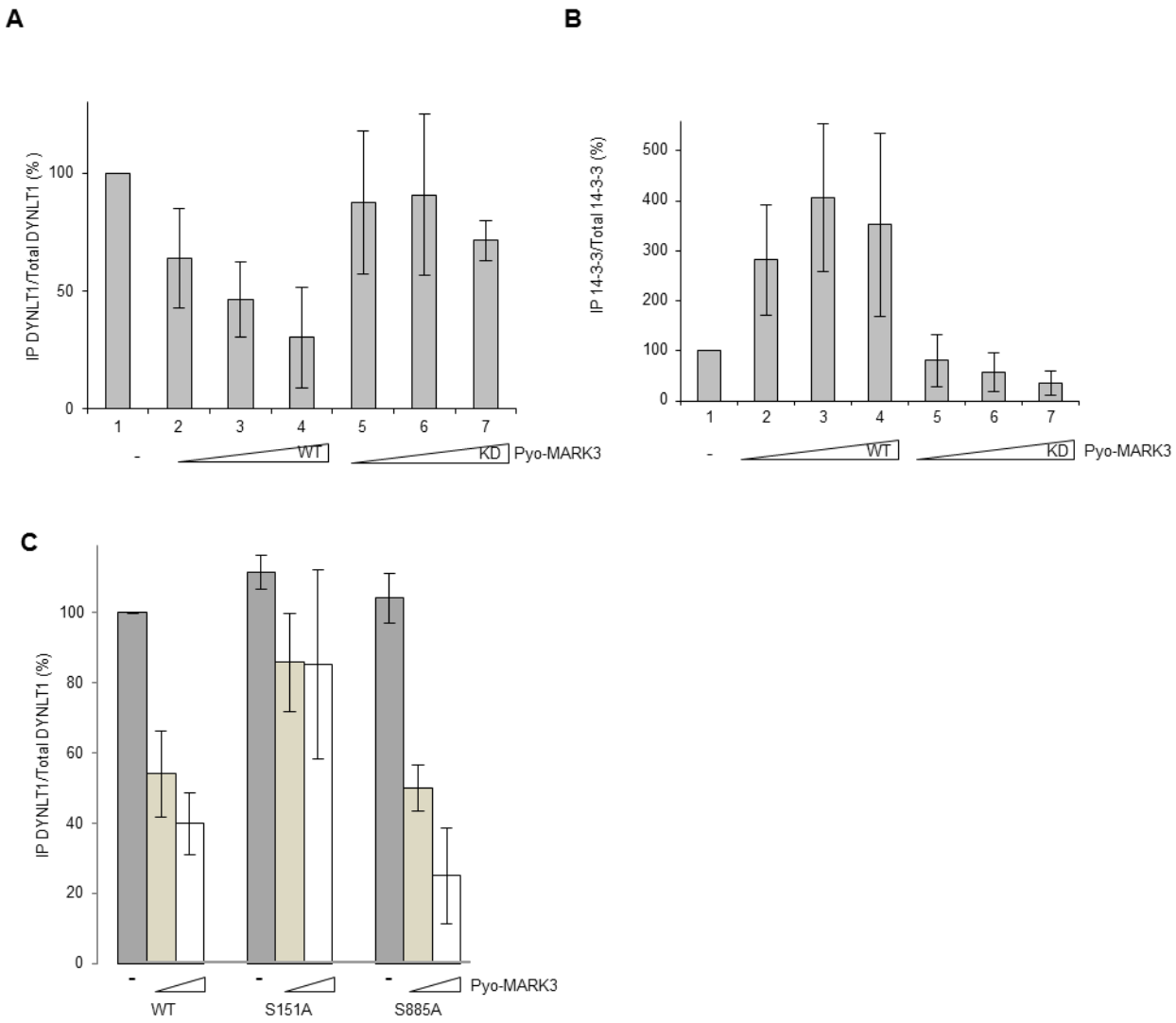

Figure S3. Quantitative analysis of the effect of MARK3 phosphorylation of ARHGEF2 on its interactions with DYNLT1 and 14-3-3.

(A, B) Quantification of the DYNLT1 and 14-3-3 interactions with ARHGEF2 in the presence of increasing wild-type MARK3 (WT) or kinase-defficient MARK3 (KD) as observed in **Figure 3C.** Data in (A) and (B) are means ± SD from four independent experiments. C) Quantification of DYNLT1 interaction with wild-type ARHGEF2 (WT) and its mutants S151A and S885A in the presence of increasing amounts of MARK3 as observed in **Figure 3D**. Data in (C) are means ± SD from three independent experiments.

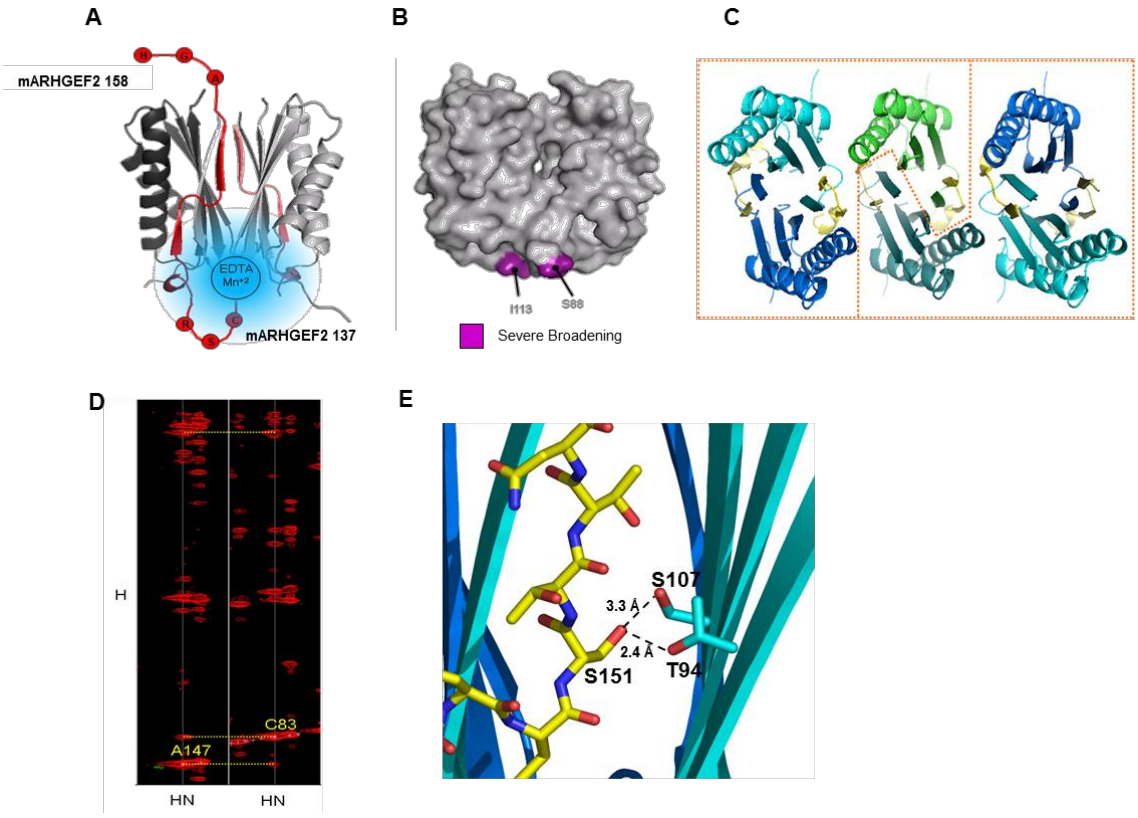

Figure S4. Structural characterization of the DYNLT1-ARHGEF2 interaction.

(A) Schematic representation of the PRE-tag conjugated to the N-terminus of the ARHGEF2 peptide (137-158). (B) Residues exhibiting substantial peak broadening induced by the addition of the PRE-tagged peptide. (C) Crystallographic contact sites of the DYNLT1-ARHGEF2 (peptides 136-164) chimera. Three molecules of DYNLT1/ARHGEF2 (136-164) chimera are present in the asymmetric unit (outlined in orange), two of which form homodimers, while the third forms a dimer with a molecule in another crystallographic symmetry unit. ARHGEF2 residues are highlighted in yellow. The asymmetric units are related to each other by the space group P3121. (D) Nuclear Overhauser Effect Spectroscopy (NOESY). Strips of the NOESY spectrum of the DYNLT1-ARHGEF2 chimera highlighting NOE connections between residues A147 and C83, consistent with the crystal structure. (E) ARHGEF2 Ser^{151} side chain hydrogen bonds with DYNLT1. Hydrogen bonds formed by the side chain hydroxyl group of ARHGEF2 Ser^{151} with the side chain hydroxyls of DYNLT1 T94 and S107 are shown as dashed lines with distances between oxygen atoms indicated.

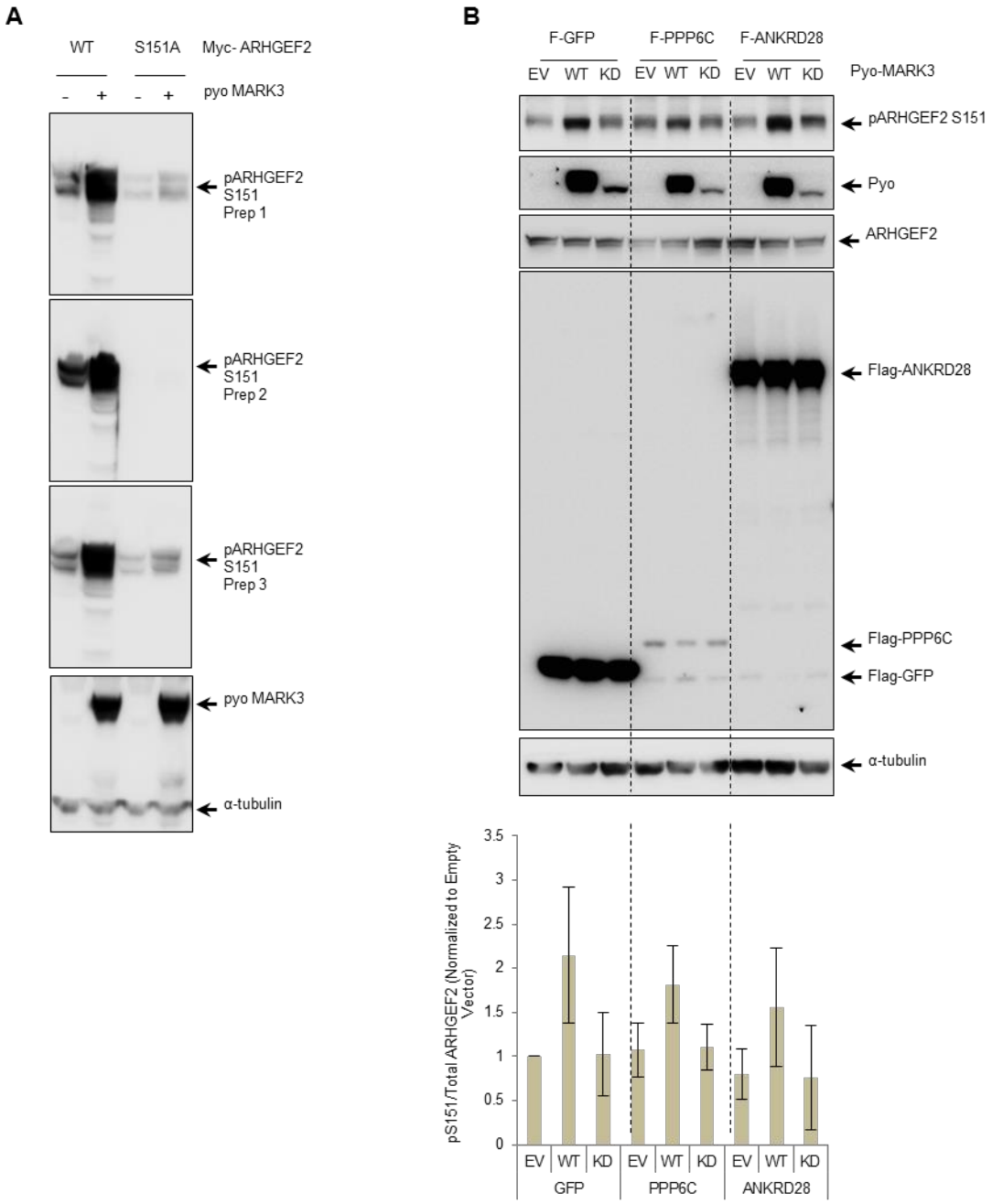

Figure S5. MARK3 but not PP6 regulates the phosphorylation of ARHGEF2 Ser^{151}.

(A) Validation of the specificity of the ARHGEF2 phospho-S151 antibody. Western blot of HEK293T overexpressing Myc-tagged wild-type ARHGEF2 (WT) or the mutant (S151A) alone or in the presence of pyo-tagged MARK3 probed with three different preparations of phospho-S151-specific antibodies (designed by Cell Signaling Technology). We retained prep 2 for our analysis. (B) Western blot of tetracycline-inducible HEK293 cell lines carrying inducible expression of Flag-tagged GFP, catalytic subunit PPP6C and regulatory subunit ANKRD28. The cells were induced overnight with tetracycline (500ng/ml) and transfected with empty vector (EV), wild type MARK3 (WT) or kinase deffcient MARK3 (KD). Endogenous ARHGEF2 phosphorylation was evaluated using the phospho-S151 specific antibodies from panel A. Data are means ± SD of four independent experiments.

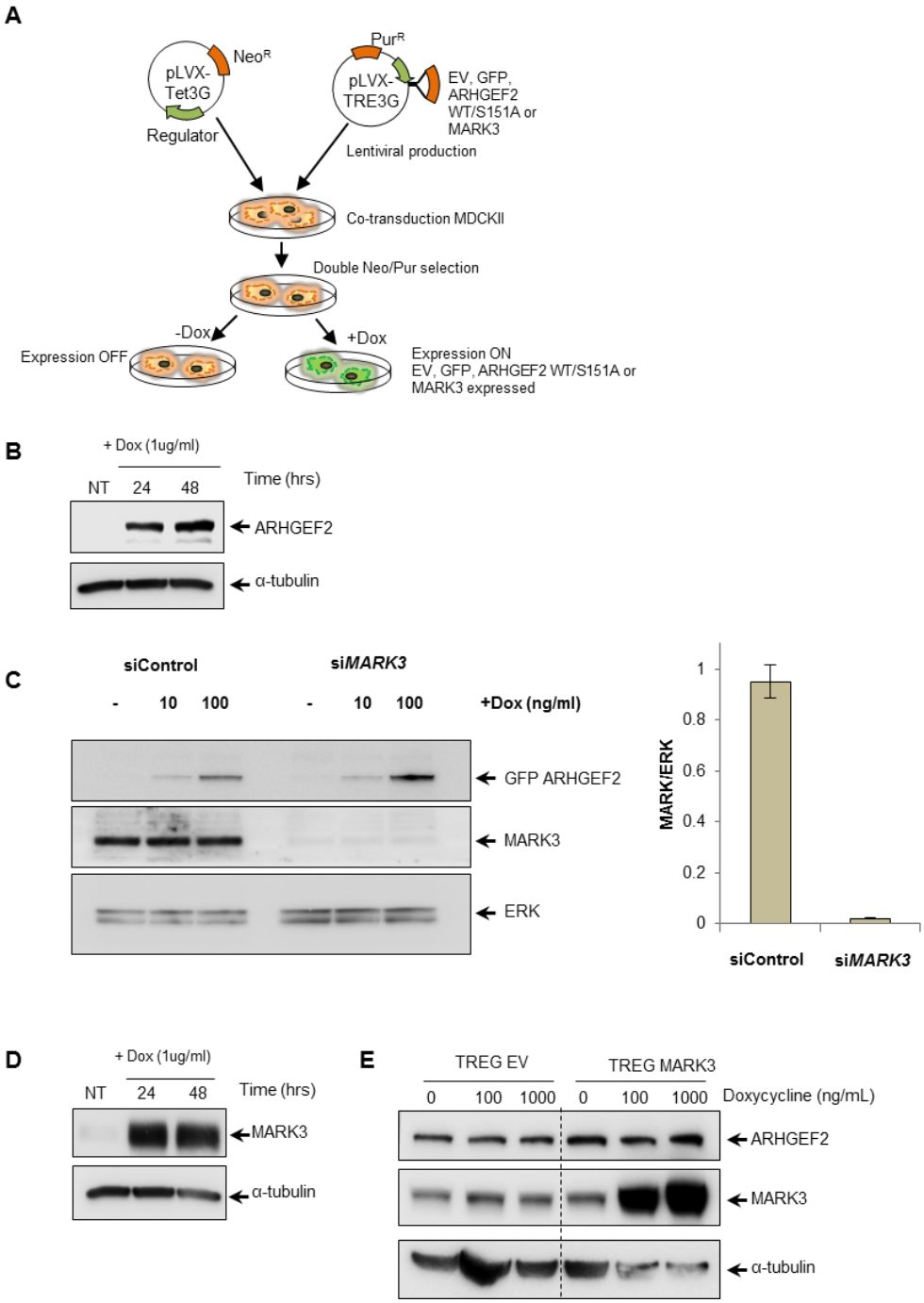

Figure S6. Generation and validation of inducible cells.

(A) Schematic of the generation process of MDCKII cells stably expressing MARK3, ARHGEF2^{WT} or ARHGEF2^{S151A} under control of a tetracycline-inducible (TETi) promoter. (B) WB of the selected colonies expressing inducible ARHGEF2. (C) WB of inducible MDCKII cells expressing pLVX-GFP ARHGEF2^{WT} with increasing doses of doxycycline treated with a control or a of specific pool of *MARK3* siRNA. Right panel: quantification of MARK3 protein is shown. Data are means ± SD of three independent experiments. (D) Validation of inducible MARK3. (E) Evaluation of the effect of EV and MARK3 induction on ARHGEF2 expression. (B, D and E. Representative of three independent experiments.

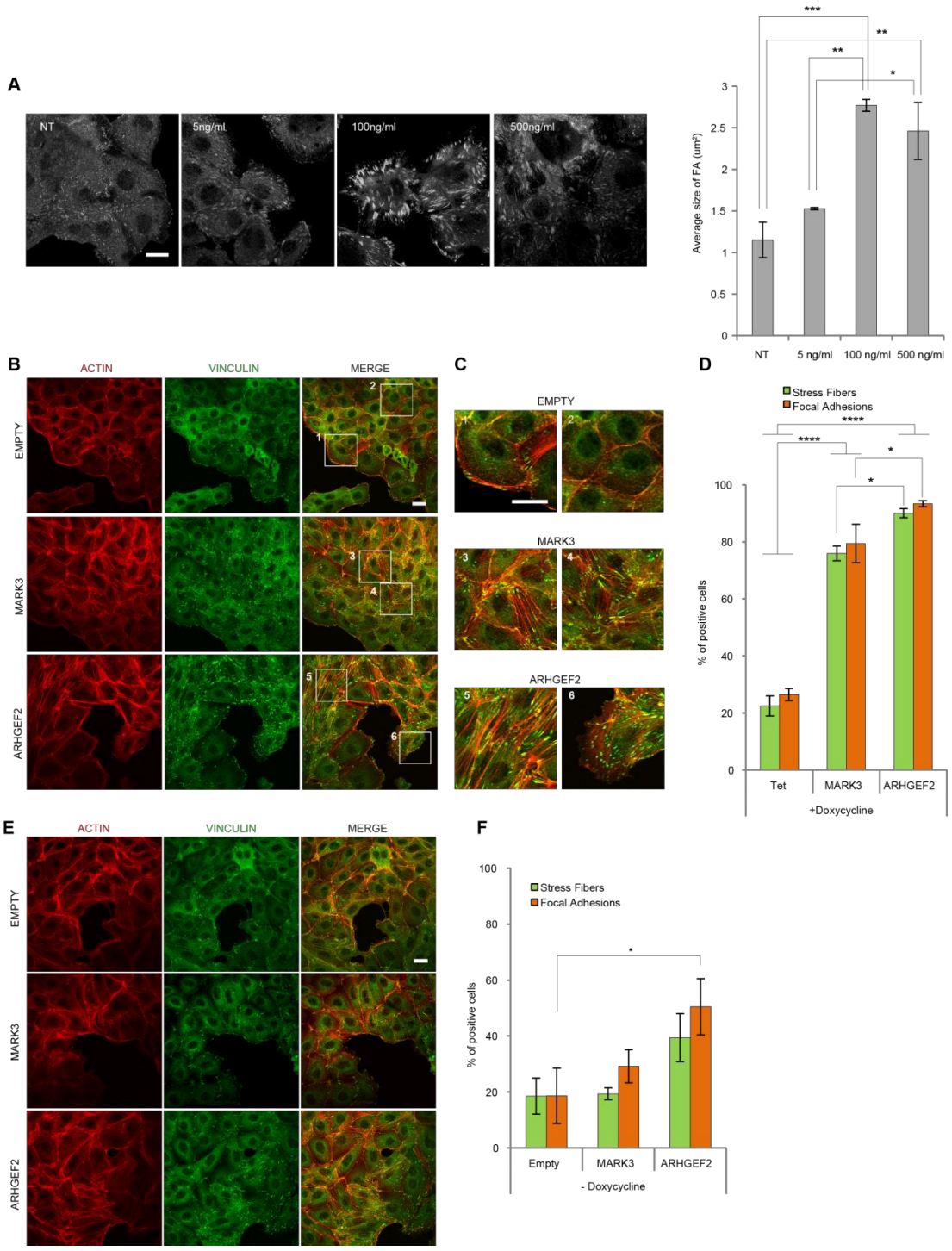

Figure S7. MARK3 affects the biological activity of ARHGEF2.

(A) Immunofluoresnce of inducible MDCKII cells expressing pLVX-GFP ARHGEF2^{WT} in the presence of increasing doses of doxycycline fixed and stained for VINCULIN. . Four high magnification fields per experiment were quantified. Data are means of two independent experiments . Scale bar, 20 µm. Statistical significance was determined by a one-way ANOVA test with a Bonferroni post-test correction for multiple comparisons. *P=0.0165; **P=0.023 (5 ng VS 100 ng) and **P=0.0017 (NT VS 500 ng); ***P=0.0003. (B) MDCKII cells expressing inducible pLVX-Empty Vector, MARK3 or wild-type ARHGEF2. Confocal images of subconfluent cells induced

with 100 ng/mL of doxycycline for 18 hours, fixed and stained for ACTIN and VINCULIN. (C) Detail of the boxed areas shown in A. (D) Quantification of stress fibers and focal adhesions of images shown in A, expressed as percent of positive cells. (E) Uninduced MDCKII as in (B), treated with DMSO 18 hours. In (F) quantification of images shown in (E). Scale bars, 20 μm. Data in (D) and (F) are means ± SD of three independent experiments, a total of n=100-200 cells per condition were counted.. Statistical significance was determined by a two-way ANOVA test with a Bonferroni post-test correction for multiple comparisons. In (D), *P=0.0136 for SF and *P=0.0143 for FA; ****P<0.0001., In (F) *P=0.0182.

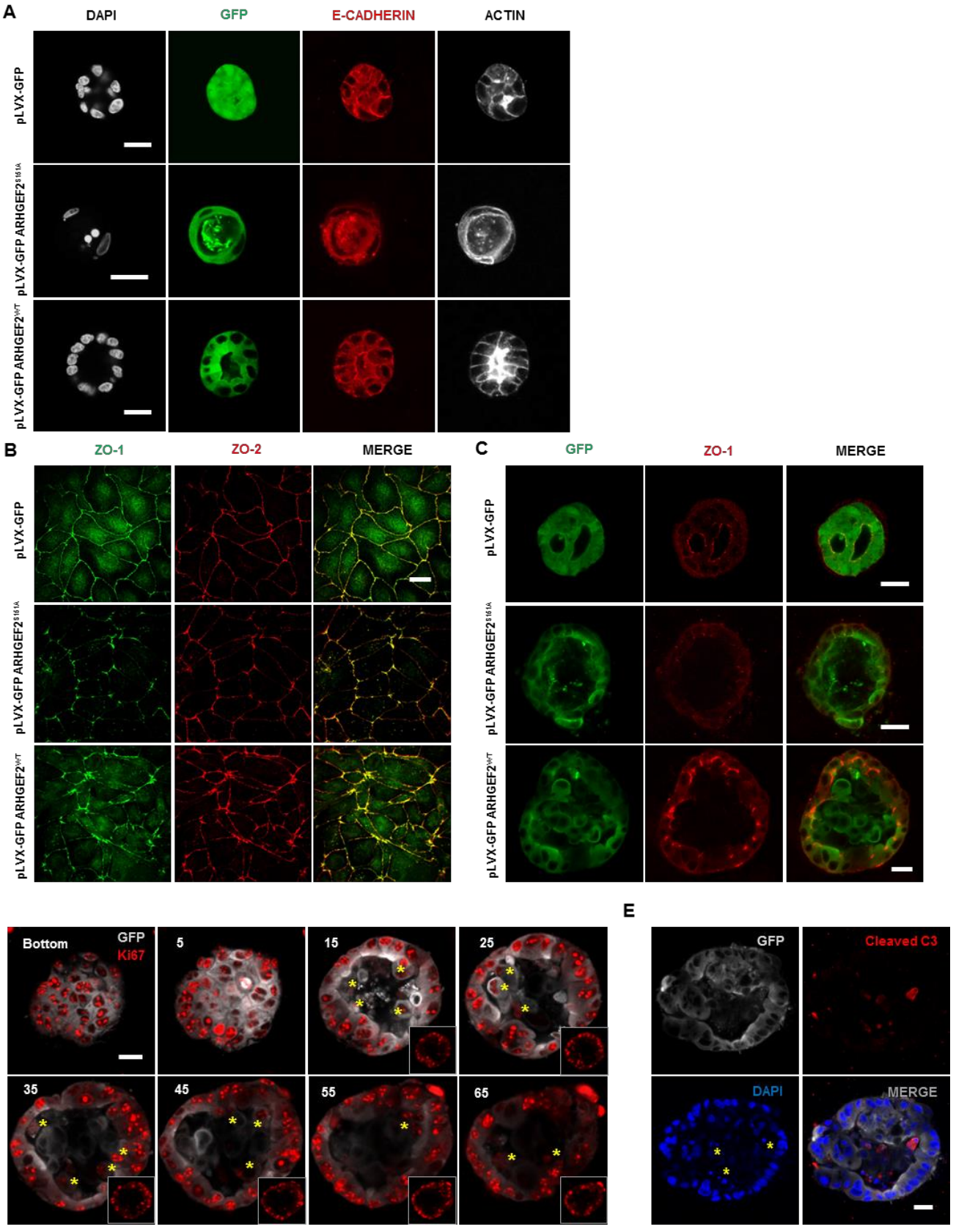
A
DAPI
GFP
E-CADHERIN
ACTIN
pLVX-GFP
pLVX-GFP ARHGEF2S161A
pLVX-GFP ARHGEF2WT
B
ZO-1
ZO-2
MERGE
C
GFP
ZO-1
MERGE
D
Bottom
GFP
Ki67
5
15
25
35
45
55
65
E
GFP
Cleaved C3
DAPI
MERGE

Figure S8. Phosphorylation of ARHGEF2 Ser^{151} is required for normal cell polarity

(A) 3D culture at four days of MDCKII cells expressing inducible pLVX-GFP, pLVX-GFP ARHGEF2^{WT} or mutant S151A. GFP fluorescence was visualized and cysts were stained for E-CADHERIN, ACTIN and DAPI. Representative of two independent experiments. Scale bar 20 μm. (B,C) Tight junction staining in MDCKII expressing pLVX-GFP, pLVX-GFP ARHGEF2^{WT} or mutant S151A. In (B), 2D staining of ZO-1 and ZO-2. In (C), 3D staining of ZO-1. Scale bar 20 um. Representative of three independent experiments. (D, E) Ki67 and cleaved caspase 3 staining in MDCKII spheroids expressing inducible pLVX-GFP ARHGEF2^{WT} at day seven. In (D), Ki67 staining through different Z stacks (the number represent the Z stack step in μm), the yellow asterisks mark the luminal cells proliferating. Representative of two independent experiments. Scale bar 20 μm. In (E), Cleaved Caspase 3 (C3) staining; the yellow asterisks mark the groups of apoptotic luminal cells that coincide with luminal nuclear fragmentation as observed in the DAPI staining. Scale bar 20 μm. Representative of two independent experiments.

Summary							FlagBirA*-ARHGEF2			
Gene ID	*Protein ID*	*Gene Nam*	*Full name*	*Top 3 controls*			*A*	*B*	*Total*	*SAINT*
9181	15011974	**ARHGEF2**	*Rho/Rac guanine nucleotide exchange factor 2*				2388	2739	**5127**	**-**
9859	109255228	**CEP170**	*centrosomal protein 170kDa*	38	35	31	236	140	**376**	**1.00**
26586	148664244	**CKAP2**	*cytoskeleton associated protein 2*	1			106	51	**157**	**1.00**
55291	255918192	**PPP6R3**	*protein phosphatase 6 regulatory subunit 3*	10	4	4	82	47	**129**	**1.00**
23243	68131557	**ANKRD28**	*ankyrin repeat domain 28*	3	1		86	42	**128**	**1.00**
79649	147903302	**MAP7D3**	*MAP7 domain containing 3*	7	7	5	68	26	**94**	**1.00**
22870	151101459	**PPP6R1**	*protein phosphatase 6 regulatory subunit 1*	4	2	1	62	28	**90**	**1.00**
54477	19923493	**PLEKHA5**	*pleckstrin homology domain containing A5*				66	21	**87**	**1.00**
283373	157743284	**ANKRD52**	*ankyrin repeat domain 52*				67	15	**82**	**1.00**
9701	37537701	**PPP6R2**	*protein phosphatase 6 regulatory subunit 2*				41	17	**58**	**1.00**
2011	254028234	**MARK2**	*microtubule affinity regulating kinase 2*	2			35	14	**49**	**1.00**
5358	209862851	**PLS3**	*plastin 3*	8	8	7	22	26	**48**	**0.90**
5537	183603929	**PPP6C**	*protein phosphatase 6 catalytic subunit*	5	3	2	32	14	**46**	**1.00**
157922	186659512	**CAMSAP1**	*calmodulin regulated spectrin associated protein 1*	2	2	1	38	6	**44**	**0.94**
22919	6912494	**MAPRE1**	*microtubule associated protein RP/EB family member 1*	6	4	4	24	19	**43**	**1.00**
220134	21450832	**SKA1**	*spindle and kinetochore associated complex subunit 1*				26	13	**39**	**1.00**
4137	8400715	**MAPT**	*microtubule associated protein tau*	1			22	16	**38**	**1.00**
4140	193083125	**MARK3**	*microtubule affinity regulating kinase 3*	4	3		25	9	**34**	**1.00**
55201	50428935	**MAP1S**	*microtubule associated protein 1S*				24	9	**33**	**1.00**
9053	310750366	**MAP7**	*microtubule associated protein 7*				23	10	**33**	**1.00**
57509	50348611	**MTUS1**	*microtubule associated tumor suppressor 1*				21	12	**33**	**1.00**
4690	5453754	**NCK1**	*NCK adaptor protein 1*	4	4	3	20	12	**32**	**0.91**
51512	253970412	**GTSE1**	*G2 and S-phase expressed 1*				24	4	**28**	**1.00**
5528	31083280	**PPP2R5D**	*protein phosphatase 2 regulatory subunit B', delta*	3	2		17	8	**25**	**0.94**
23271	44955929	**CAMSAP2**	*calmodulin regulated spectrin associated protein family member 2*				20	4	**24**	**1.00**
256714	270483740	**MAP7D2**	*MAP7 domain containing 2*				17	4	**21**	**1.00**
10298	5031975	**PAK4**	*p21 protein (Cdc42/Rac)-activated kinase 4*	4	2	1	10	8	**18**	**0.79**
9201	4758128	**DCLK1**	*doublecortin like kinase 1*	2			9	7	**16**	**1.00**
51248	7706025	**PDZD11**	*PDZ domain containing 11*				9	6	**15**	**1.00**
9928	7661878	**KIF14**	*kinesin family member 14*	1			11	3	**14**	**0.99**
283638	163644261	**KIAA0284**	*centrosomal protein 170B*				8	4	**12**	**1.00**
79884	88759339	**MAP9**	*microtubule associated protein 9*				8	4	**12**	**1.00**
23332	214010173	**CLASP1**	*cytoplasmic linker associated protein 1*				9	3	**12**	**0.99**
1855	32479521	**DVL1**	*dishevelled segment polarity protein 1*				5	4	**9**	**1.00**
121441	206597465	**NEDD1**	*neural precursor cell expressed, developmentally down-regulated 1*	1			5	4	**9**	**1.00**
6729	226371618	**SRP54**	*signal recognition particle 54kDa*	1			4	3	**7**	**0.99**

Table S1. ARHGEF2 interactors reported in this study –Summary.
Summary of the BioID analysis for ARHGEF2. The spectral counts of the top 3 controls out of 14 are shown. "A" and "B" denote two independent biological replicates. "Total" is the sum of the spectral counts for the prey. Bait Spectral Counts are highlighted in yellow. SAINT or significant analysis interactome score >0.79 was used to identified *bona fide* interactors.

GO biological process complete	Accession number	Homo sapiens - REFLIST (20972)	upload_1 (35)	upload_1 (expected)	upload_1 (over/under)	upload_1 (fold Enrichment)	upload_1 (P-value)	-Log_{10} P-value
microtubule cytoskeleton organization	GO:0000226	397	12	0.66	+	18.11	9.90E-09	8.004365
cytoskeleton organization	GO:0007010	960	16	1.6	+	9.99	5.50E-09	8.259637
single-organism organelle organization	GO:1902589	1547	16	2.58	+	6.2	6.70E-06	5.173925
microtubule-based process	GO:0007017	574	13	0.96	+	13.57	3.41E-08	7.467246
regulation of microtubule cytoskeleton organization	GO:0070507	129	5	0.22	+	23.22	2.06E-02	1.686133
regulation of microtubule polymerization or depolymerization	GO:0031110	56	5	0.09	+	53.5	3.46E-04	3.460924
regulation of microtubule-based process	GO:0032886	153	5	0.26	+	19.58	4.70E-02	1.327902
negative regulation of microtubule binding	GO:1904527	2	2	0	+	> 100	4.54E-02	1.342944
COPII vesicle coating	GO:0048208	61	4	0.1	+	39.29	2.93E-02	1.533132
COPII-coated vesicle budding	GO:0090114	64	4	0.11	+	37.45	3.54E-02	1.450997
cellular localization	GO:0051641	2131	15	3.56	+	4.22	4.73E-03	2.325139
organelle organization	GO:0006996	2952	23	4.93	+	4.67	3.21E-08	7.493495
cellular component organization	GO:0016043	5382	26	8.98	+	2.89	2.03E-05	4.692504
cellular component organization or biogenesis	GO:0071840	5548	26	9.26	+	2.81	4.09E-05	4.388277
vesicle coating	GO:0006901	63	4	0.11	+	38.04	3.33E-02	1.477556
protein complex subunit organization	GO:0071822	1205	11	2.01	+	5.47	2.17E-02	1.663540
vesicle targeting, rough ER to cis-Golgi	GO:0048207	61	4	0.1	+	39.29	2.93E-02	1.533132
vesicle targeting, to, from or within Golgi	GO:0048199	65	4	0.11	+	36.87	3.76E-02	1.424812
vesicle targeting	GO:0006903	77	5	0.13	+	38.91	1.66E-03	2.779892
organelle localization	GO:0051640	462	9	0.77	+	11.67	4.31E-04	3.365523
establishment of organelle localization	GO:0051656	395	8	0.66	+	12.14	1.99E-03	2.701147
mitotic cell cycle process	GO:1903047	761	9	1.27	+	7.09	2.74E-02	1.562249
mitotic cell cycle	GO:0000278	789	10	1.32	+	7.59	3.68E-03	2.434152
establishment or maintenance of cell polarity	GO:0007163	154	5	0.26	+	19.45	4.85E-02	1.314258
Unclassified	UNCLASSIFIED	3875	5	6.47	-	0.77	0.00E+00	
Analysis Type:	PANTHER Overrepresentation Test (release 20160715)							
Annotation Version and Release Date:	GO Ontology database Released 2016-12-28							
Analyzed List:	upload_1 (Homo sapiens)							
Reference List:	Homo sapiens (all genes in database)							
Bonferroni correction:	TRUE							
Bonferroni count:	8404							

Table S2. GO enrichment analysis of the ARHGEF2 network.
Enrichment analysis of the ARHGEF2 network, based on biological process, performed using http://www.geneontology.org/. The analysis results are sorted hierarchically, based on their ontology. The most specific subclass first, with its parent terms indented directly below it. ARHGEF2 was excluded from the analysis. – Log_{10} of the P value was calculated and used to generate the graph in Figure S1B.

Gene ID	Gene Name	Saint Score	Previously Validated	Function
23243	**ANKRD28**	**1**	Yes	Putative regulatory subunit of protein phosphatase 6, involved in mitosis and chromosome segregation.
283373	**ANKRD52**	**1**		Putative regulatory subunit of protein phosphatase 6, involved in mitosis and chromosome segregation.
157922	**CAMSAP1**	**0.94**		Regulation of cell morphology and cytoskeletal organization. Microtubule stabilization.
23271	**CAMSAP2**	**1**		Microtubule minus-end binding protein that may regulate the organization of non-centrosomal microtubules. Microtubule stabilization.
9859	**CEP170**	**1**	Yes	Centrosomal protein. Microtubule organization and cell morphology.
283638	**CEP170B (KIAA0284)**	**1**		Centrosomal protein. Microtubule organization.
26586	**CKAP2**	**1**		Stabalizes microtubules and plays a role in the regulation of cell division.
23332	**CLASP1**	**0.99**		Microtubule plus-end tracking protein that promotes the stabilization of dynamic microtubules. Required for the polarization of the cytoplasmic microtubule arrays in migrating cells towards the leading edge of the cell.
9201	**DCLK1**	**1**		Involved in a calcium- signaling pathway controlling neuronal migration in the developing brain.
1855	**DVL1**	**1**		Regulates cell proliferation, acting as a transducer molecule for developmental processes, including segmentation and neuroblast specification.
51512	**GTSE1**	**1**		May be involved in p53-induced cell cycle arrest in G2/M phase by interfering with microtubule rearrangements that are required to enter mitosis.
9928	**KIF14**	**0.99**		Plays an essential role in cytokinesis.
55201	**MAP1S**	**1**		Microtubule-associated protein that mediates aggregation of mitochondria resulting in cell death and genomic destruction. Plays a role in anchoring the microtubule organizing center to the centrosomes. Binds to DNA. Plays a role in apoptosis. Involved in the formation of microtubule bundles
9053	**MAP7**	**1**		Microtubule-stabilizing protein that may play an important role during reorganization of microtubules during polarization and differentiation of epithelial cells. Associates with microtubules in a dynamic manner. May play a role in the formation of intercellular contacts.
256714	**MAP7D2**	**1**		Involved in microtubule stabilization.
79649	**MAP7D3**	**1**		Promotes the assembly and stability of microtubules
79884	**MAP9**	**1**		Involved in organization of the bipolar mitotic spindle. Required for bipolar spindle assembly, mitosis progression and cytokinesis. May act by stabilizing interphase microtubules.
22919	**MAPRE1**	**1**		Binds to the plus end of microtubules and regulates the dynamics of the microtubule cytoskeleton. Promotes cytoplasmic microtubule nucleation and elongation. May be involved in spindle function by stabilizing microtubules and anchoring them at centrosomes. May play a role in cell migration.
4137	**MAPT**	**1**		Promotes microtubule assembly and stability, and might be involved in the establishment and maintenance of neuronal polarity.
2011	**MARK2**	**1**	Yes	Involved in cell polarity and microtubule dynamics regulation. Plays a key role in cell polarity by phosphorylating the microtubule-associated proteins MAP2, MAP4 and MAPT/TAU at KXGS motifs, causing detachment from microtubules, and their disassembly.
4140	**MARK3**	**1**		Involved in the specific phosphorylation of microtubule- associated proteins for tau, MAP2 and MAP4.
57509	**MTUS1**	**1**		Microtubule associated tumor suppressor 1. Cooperates with AGTR2 to inhibit ERK2 activation and cell proliferation.
4690	**NCK1**	**0.91**		Adaptor protein. Plays a role in ELK1-dependent transcriptional activation in response to activated Ras signaling. Involved in actin polymerization.
121441	**NEDD1**	**1**		Required for mitosis progression. Promotes the nucleation of microtubules from the spindle.
10298	**PAK4**	**0.79**	Yes	Serine-threonine kinase. Involved in cytoskeleton regulation, cell migration, growth, proliferation or cell survival and affects actin cytoskelton reorganization.
51248	**PDZD11**	**1**		Involved in biotin and ion transmembrane transport.
54477	**PLEKHA5**	**1**		Phosphatidylinositol-3-phosphate and phosphatidylinositol-3,5-bisphosphate binding. Involved in reproductive system and brain development.
5358	**PLS3**	**0.9**		Actin-bundling protein. Involved in actin and calcium ion binding.
5528	**PPP2R5D**	**0.94**		Implicated in the negative control of cell growth and division.
5537	**PPP6C**	**1**	Yes	Catalytic subunit of protein phosphatase 6. Component of a signaling pathway regulating cell cycle progression, mitotic spindle formation and chromosome segreagation.
22870	**PPP6R1**	**1**	Yes	Regulatory subunit of protein phosphatase 6. May function as a scaffolding PP6 subunit.
9701	**PPP6R2**	**1**		Regulatory subunit of protein phosphatase 6. May function as a scaffolding PP6 subunit.
55291	**PPP6R3**	**1**		Regulatory subunit of protein phosphatase 6. May function as a scaffolding PP6 subunit.
220134	**SKA1**	**1**		Component of the SKA1 complex, a microtubule-binding subcomplex of the outer kinetochore that is essential for proper chromosome segregation.
6729	**SRP54**	**0.99**		Binds to the signal sequence of presecretory protein when they emerge from the ribosomes. RNA and GTP binding

Table S3. Functional Annotation of ARHGEF2 interactors.
Functional annotation was made based on GO analysis and a survey of the literature. Saint score and previously validated interactors are indicated.

		ANALYSIS OF MARK3/C-TAK1 COMPLEXES				
Protein Name	**ID (Uniprot)**	**Gene Name**	**Known Binding**	**MARK3* (*Ave. # of peptides)**	**Control* (*Ave. # of peptides)**	**Comments**
MARK3/C-TAK1	P27448	MARK3		358	0	Member of CAMK Ser/Thr protein kinase family, MARK subfamily. 1 KA1 Domain. 1 UBA Domain
14-3-3 FAMILY MEMBERS						
14-3-3 protein epsilon	P62258	YWHAE	Yes	133 (+131)	2	Belongs to the 14-3-3 family.
14-3-3 protein zeta/delta	P63104	YWHAZ	Yes	72 (+69)	3	Belongs to the 14-3-3 family.
14-3-3 protein theta	P27348	YWHAQ	Yes	37(+35)	2	Belongs to the 14-3-3 family.
14-3-3 protein eta	Q04917	YWHAH	Yes	33(+34)	1	Belongs to the 14-3-3 family.
14-3-3 protein gamma	P61981	YWHAG	Yes	42(+40)	2	Belongs to the 14-3-3 family.
14-3-3 protein beta	P31946	YWHAB	Yes	60(+51)	9	Belongs to the 14-3-3 family.
MICROTUBULE-ASSOCIATED						
CLIP-associating protein 2	O75122	CLASP2		42	0	Microtubule plus-end tracking protein that promotes the stabilization of dynamic microtubules. LqRsrSdidV
CLIP-associating protein 1	Q7Z460	CLASP1		32	0	Microtubule plus-end tracking protein that promotes the stabilization of dynamic microtubules. LqRsrSdidV
GEF-H1, Rho/Rac guanine nucleotide exchange factor 2	Q92974	ARHGEF2		28	0	Activates Rho-GTPases by promoting the exchange of GDP for GTP. Localizes to the tips of cortical microtubules of the mitotic spindle during cell division, and is further released upon microtubule depolymerization.
Cytoskeleton-associated protein 5	Q14008	CKAP5		27(+21)	6	Plays a major role in organizing spindle poles. LaReaStgvL
MAP4, Microtubule-associated protein 4 (MAP 4)	P27816	MAP4	Yes	26(+17)	9	Non-neuronal microtubule-associated protein. Promotes microtubule assembly.
Kinesin-2	O00139	KIF2A		13(+10)	3	Plus end-directed microtubule-dependent motor required for normal brain development. Required for normal progression and spindle dynamics during mitosis.
MAP7, Microtubule-associated protein 7	Q14244	MAP7		5	0	Microtubule-stabilizing protein that may play an important role during reorganization of microtubules during polarization and differentiation of epithelial cells.
Microtubule-actin cross-linking factor 1, isoforms 1/2/3/5	Q9UPN3	MACF1		2	0	F-actin-binding protein which may play a role in cross-linking actin to other cytoskeletal proteins. Also binds to microtubules. VeKrrSIIeI, VqKafSidiI
Microtubule-associated protein RP/EB family member 3, RP3	Q9UPY8	MAPRE3		2	0	May be involved in microtubule polymerization, and spindle function by stabilizing microtubules and anchoring them at centrosomes. May play a role in cell migration
SIGNALING RELATED						
TBC1 domain family member 4	O60343	TBC1D4		30(+29)	1	May act as a GTPase-activating protein for RAB2A, RAB8A, RAB10 and RAB14.
SH2 containing inositol-5-phosphatase	O00145	INPP5D		10	0	Phosphatidylinositol (PtdIns) phosphatase that specifically hydrolyzes the 5-phosphate of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P3) to produce PtdIns(3,4)P2, thereby negatively regulating the PI3K (phosphoinositide 3-kinase) pathways.
PP2A, subunit A, PR65-alpha isoform	P30153	PPP2R1A		9(+8)	1	Serine/threonine protein phosphatase PP2A scaffolding subunit A
PP2A, subunit B, B-alpha isoform	P63151	PPP2R2A		3	0	Serine/threonine protein phosphatase PP2A regulatory subunit B family.
PP2C gamma	O15355	PPM1G		8(+7)	1	Serine/threonine protein phosphatase PP2C catalytic subunit
PRAS40	Q96B36	AKT1S1		4	0	Subunit of TORC1
Tyrosine-protein phosphatase, non-receptor type 13	Q12923	PTPN13		3	0	Tyrosine phosphatase which regulates negatively FAS-induced apoptosis and NGFR-mediated pro-apoptotic signaling. MgRaiStgsL, IlKrlScseL, IsKvpStPvhL
Tyrosine-protein phosphatase, non-receptor type 14	Q15678	PTPN14		3	0	Tyrosine phosphatase

Table S4. Analysis of MARK3/C-TAK1 complexes.
Summary of the different interactors found for MARK3. Previously known binding interactors are indicated. Ave.: Average.

Data Collection Statistics:	
Space group	$P\,3_2\,2\,1$
Cell dimensions	
a, b, c (Å)	91.97, 91.97, 83.08
α, β, γ (°)	90, 90, 120
Resolution (Å)	50–2.00 (2.03–2.00)[a]
Rmerge	0.078 (0.348)
$I/\sigma I$	31.5 (6.6)
Completeness (%)	100 (100)
Redundancy	10.8 (9.2)
Refinement Statistics:	
Rwork	17.4%
Rfree	21.0%
No. of Reflectctions	27423
No. of Reflections used in Rfree	2010

[a] Values in parentheses are for the highest resolution shell.

Table S5: Data collection and refinement statistics for DYNLT1:ARHGEF2 chimera (PDB: 5WI4).