## SUPPLEMENTARY MATERIAL

## Methods

Plasmids - Caenorhabditis elegans small G protein constructs were either PCR cloned from a *C. elegans* cDNA library (*de novo*) or retrieved from the *C. elegans* ORFeome collection version 1.1 {Reboul, 2003 #12} using Gateway® cloning technology (Invitrogen) as described previously {Caruso, 2005 #10}. Following manufacturer's instructions, amplified ORFs were recombined using BP clonase into Gateway pDONR201 vectors (Invitrogen). This provided entry clones that were subsequently used to transfer the target gene in three different Gateway compatible expression vectors using LR clonase i) pGEX-2TK, a bacterial expression vector to produce N-terminally tagged GST fusion proteins required for the AlphaScreen GTP affinity; ii) pL4440, to perform RNAi by feeding; and iii) pECFP, a mammalian expression vector producing fluorescent proteins suitable for analysis of the subcellular localization of the recombinant protein.

Alphascreen – Recombinant GST-GTPases were expressed in bacteria and purified using glutathione Sepharose 4B as described previously {Caruso, 2005 #10}. During the purification step, GST-GTPases were unloaded from their intrinsic nucleotide by adding 5 mM EDTA in lysate before purification. AlphaScreen competition assays were then performed as described previously {Caruso, 2005 #10}. Briefly, nucleotide-free GST-GTPases were incubated with 30 nM biotin-GTPγS and increasing concentrations of unlabeled GTP (ranging from 0.4 nM to 3 μM) for 30 minutes in a 384-well plate. Streptavidin Donor beads and GSH Acceptor beads (PerkinElmer) were added simultaneously at a final concentration of 0.02 mg/ml per well and incubated for 1 h at 23°C before reading. Laser excitations were carried out at 680 nm, and readings were performed at 520–620 nm using an Envision plate reader (PerkinElmer). The IC50 value corresponds to the competitor concentration required to reduce the maximal signal (obtained in the absence of competitor) by 50%. The IC50 values for the competition assay represent the average of at least three independent experiments each performed in triplicate. ND, not determined.

Sub-cellular localization and siRNA treatments in mammalian cells - Cellular localization was assessed by transfecting ECFP-tagged small G protein constructs

into Vero cells (ATCC CCL81). Transfections and live cell imaging were carried out as described previously {Simpson, 2000 #13}. Downregulation of G proteins was carried out in reverse transfection format. Briefly, siRNAs (Ambion) were mixed with Lipofectamine2000 transfection reagent (Invitrogen) and printed on to chambered glass coverslips. Vero cells were added to these siRNA arrays and gene downregulation allowed to proceed for 32 hours. Following this, cells were infected with recombinant adenoviruses expressing ECFP-tsO45G for 16 hours at 39.5°C in order to accumulate the tsO45G cargo in the ER. A pulse of tsO45G was then released through the secretory pathway at 32°C for 1 hour, and the cells were fixed and immunostained for cell surface tsO45G. Secretory activity of the cells was calculated from the ratio of cell surface tsO45G to total tsO45G as described previously {Simpson, 2007 #14}. Two independent siRNAs were used to target each small G protein.

*Tissue localization* - Expression profiles were analyzed using GFP-reporter transgenic worms {Hunt-Newbury, 2007 #15}. Living worms were mounted on agarose pads in M9 buffer containing 1mM Levamisol (Sigma). Fluorescence emission from living worms was observed using an Axiovert-200 microscope (Zeiss) and images analyzed using Northern Eclipse V6.0 (Empix Imaging, Mississauga, Ontario, Canada).

*RNAi in C. elegans* - RNAi experiments were carried out using a feeding procedure as described previously {Caruso, 2008 #11}. pL4440 vector containing GTPases gene were transformed into HT115(DE3) bacteria. Isolated bacterial clones were grown for 12h at 37°C in Luria-Bertani (LB) medium supplemented by  $100\,\mu$  g/ml ampicillin. Bacteria were spotted on NGM plates containing 1mM IPTG and  $100\,\mu$  g/ml ampicillin, dried and induced overnight at room temperature. Synchronized L1 worm populations were transferred to these plates and treated for three days. L4 worms were then analyzed under optical microscope for phenotypic change compare to untreated control worms.

Messenger RNA Co-expression - mRNA co-expression data were retrieved for Wormbase and corresponded to data generated previously {Kim, 2001 #20}.

Hierarchical clustering and Network analyses - To integrate data, a matrix combination approach was undertaken (||A-B||). Matrices were computed from the alignment using PHYLIP ProtDist according to the Jones-Taylor-Thornton model. The hierarchical clustering tree was computed using the PHYLIP Neighbor command based on the UPGMA method and visualized using TreeView. Biochemical values were used to generate distance matrices based on biochemical activity(ies) similarities (alone or in combination). These matrices were calculated based on Euclidian distances, and hierarchical clustering trees were then computed using the PHYLIP Neighbor command based on the UPGMA method and visualized using TreeView. Tree comparison was carried out using PHYLIP Treedist. The amino acid sequences corresponding to these genes were retrieved for Wormbase database (http://www.wormbase.org/). Sequence-based hierarchical clustering was performed using ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html). All the clustering data analyses were carried out usina the PermutMatrix software (http://www.lirmm.fr/~caraux/PermutMatrix/) and network analyses were performed using Cytoscape (http://www.cytoscape.org/).

**Table S1**: *C. elegans* small GTPases and their human closest counterpart. Common and WormBase sequence names of the 59 small G proteins identified in *C. elegans* genome with the cloning strategy used and the expression vectors in which the corresponding gene was successfully cloned as well as their function in *C. elegans* when elucidated.

when	elucida	ated.						
Sub-		WormBase	Closest	Cloning	pGEX-			
family	Name	sequence	human	Cloning	PGEX- 2TK	pECFP	pL4440	Function in C.elegans
ramily		name	homolog	strategy	ZIK			
	Crp-1	Y32F6B.3		De novo	Х	Х	х	Regulation of the UPR, membrane trafficking
	Rac-2	K03D3.10	Rac2	ORFeome	X	X	X	CAN and PDE axon pathfinding, cell migration
5	Cdc-42	R07G3.1	Cdc42	ORFeome	X	X	X	Control embryonic polarity
Rho (7)	Rho-1	Y51H4A.3	RhoA	ORFeome	X	X	X	Emb polarity, cell mugration, cell shape, muscle contraction
듄	Chw-1	F22E12.2	RhoU	De novo				Emb pounty, con magnation, con emape, massic contraction
	Ced-10	C09G12.8	Rac1	ORFeome	Х	X	Х	Phagocytosis in apoptosis, migration of distal tip cells
	Mig-2	C35C5.4	RhoG	ORFeome	X	X	X	Cell migration, axon outgrowth and guidance
_	mig 2		TAILOG	OKI COMC		^		Spindle formation, centrosome connection, nuclear envelope
돌	Ran	K01G5.4	Ran1	ORFeome	X	X	X	assembly
	rab-1	C39F7.4	Rab1	ORFeome	Х	X	X	Intracellular vesicule trafficking, fertility, movement, and survival
	unc-108	F53F10.4	Rab2	ORFeome	X	X	X	Removal of apoptotic cells, neurons locomotion
	rab-3	C18A3.6	rab3	ORFeome	X	X	X	Synaptic transmission, exocytosis
	rab-5	F26H9.6	Rab5	De novo	X	X	X	Membrane fusion, ER morphology, fat storage
	rab-6.1	F59B2.7	Rab6	De novo	X	X	X	Embryonic development and reproduction
	rab-6.2	T25G12.4	rab6	De novo	X	X	X	Emoryonic development and reproduction
	rab-7	W03C9.3	Rab7	De novo	^	X	X	Vesicular trafficking, regulation of fat storage
	rab-8	D1037.4	Rab8	De novo	Х	X	X	Affect ovarian morphology and function
	rab-8 rab-10	T23H2.5	Rab10	ORFeome	X	_	X	Basolateral endocytic recycling in the intestine
	rab-10	F53G12.1				X	X	
	Rab-11.1	W04G5.2	Rab11	ORFeome ORFeome	X	X	X	Cytokinesis during early embryogenesis, uptake of yolk proteins
		K09A9.2	Dob44		_	_		
	rab-14		Rab14	ORFeome	X	X	X	
6	rab-18	Y92C3B.3	Rab18	De novo	X	X		
Ran (29)	rab-19	Y62E10A.9	Rab19	ORFeome	X	X	X	
2	rab-21	T01B7.3	Rab21	ORFeome	X	X	X	to the total and
	aex-6	Y87G2A.4	Rab27	ORFeome	X	X	X	Insulin branch or the dauer pathway
	rab-28	Y11D7A.4	Rab28	ORFeome	X	X	X	
	rab-30	Y45F3A.2	Rab30	ORFeome	Х	X	X	
	rab-33	F43D9.2	Rab33B			X	X	
	rab-35	Y47D3A.25	Rab35	ORFeome	Х	X	X	
	rab-37	W01H2.3	Rab37	De novo	X	X	X	
	rab-39	D2013.1	Rab39	ORFeome	Х	X	Х	
	tag-312	C33D12.6	RASEF1	De novo		X		
		K02E10.1	====	De novo				
		4R79.2	EFCAB4B					
		C56E6.2	Rab6A	De novo				
		F11A5.3	Rab2	ORFeome	X	X	X	
		F11A5.4	Rab2A	ORFeome	X	X	X	
	ras-1	C44C11.1	R-Ras2	De novo	X	X	X	MAK pathway, aging, vulva development
	ras-2	F17C8.4	M-ras	De novo	X	X	X	
	let-60	ZK792.6	K-ras	ORFeome	X	X	X	
6	rap-1	C27B7.8	Rap1b	ORFeome	X	X	X	Epithelial integrity
Ras	rap-2	C25D7.7	Rap2a	ORFeome	X	X	X	Epithelial integrity
~	rap-3	C08F8.7	Rap1c	De novo				
	Rheb-1	F54C8.5	RheB	De novo	X	X	X	
	drn-1	C54A12.4	Di-ras2	ORFeome				
	ral-1	Y53G8AR.3	RalA	ORFeome	X	X	Х	
	arf-1.1	F45E4.1	Arf1	ORFeome	X	X	X	
	arf-1.2	B0336.2.1	Arf1.2	De novo	X	X	X	Regulate membrane traffic and the actin cytoskeleton
	arf-3	F57H12.1	Arf5	De novo	X	X	X	
	arf-6	Y116A8C.12		ORFeome	X	X	X	Regulate receptor-mediated endocytosis
•	arl-1	F54C9.10	Arf1	ORFeome	X	X	X	
3	evl-20	F22B5.1	Arl2	ORFeome	X	X		Cortical microtubule formation
Sar	arl-3	F19H8.3	Arl3	ORFeome	X	X	X	
Aff/Aft/Sar (14)	arl-5	ZK632.8	Arl5	ORFeome	X	X	X	
£	arl-6	C38D4.8	Arl6	ORFeome	X	X	X	
4	arl-8	Y57G11C.13	Arl8b	De novo		X	X	
	sar-1	ZK180.4	Sar1	De novo		X	X	
	arc-1	ZK1320.6	Mid1	De novo				
		Y54E10BR.2		De novo		X	X	
		Y37E3.5		De novo		X	X	
			_					

**Table S2**: *C. elegans* small **G** protein cellular localization. Cellular localizations were determined using ECFP tagged GTPases transfected into Vero cells and visualized using fluorescence microscopy. Empty slots correspond to localizations that were not determined.

C elegans gene name	Gene designation (WormBase)			Ce	llula	ar lo	cali	zati	on		
		Nuclear membrane	ER (minor Golgi)	Intermediate compartment	Golgi	recycling endosome, endo. vesicle	Endocytic vesicules	Microtubules	Plasma membrane	Diffuse cytoplasm	Nucleus
rab-1	C39F7.4	0	0	0	1	0	0	0	0	0	0
rab-2/unc-108	F53F10.4	0	0	0	1	0	0	0	0	0	0
rab-3	C18A3.6	0	0	0	0	0	0	1	1	0	0
rab-5	F26H9.6	0	0	00	0	0	0	0	0	0	1 0
rab-6.1 rab-6.2	F59B2.7 T25G12.4	0	0	0	1	0	0	0	0	1	0
rab7	W03C9.3	0	1	0	0	0	0	0	0	0	0
rab-8	D1037.4	0	1	0	0	0	0	0	1	0	0
rab-10	Т23Н2.5	0	1	0	1	0	1	1	0	0	0
rab11.1	F53G12.1	0	0	0	0	1	0	0	1	0	0
rab14	K09A9.2	0	0	0	1	0	1	0	0	0	0
rab-18a	Y92C3B.3	0	0	0	0	0	0	0	0	1	1
rab-19	Y62E10A.9	0	1	0	1	0	0	0	0	0	0
rab-21	T01B7.3	0		0	0	0	1	0	0	0	0
aex-6	Y87G2A.4	0	1	0	0	0	0	0	0	0	0
rab-28	Y11D7A.4	0	1	0	0	0	0	0	0	0	0
rab-30	Y45F3A.2	0	0	0	1	0	0	0	0	0	0
rab-33	F43D9.2	0	0	0	1	0	0	0	0	0	0
rab-35	Y47D3A.25	0	0	0	0	1	0	0	1	0	0
rab-37	W01H2.3	0	0	0	0	0	1	0	0	0	0
rab-39	D2013.1	0	0	0	0	0	0	0	0	1	1
rabY1/tag312	C33D12.6	0	0	0	0	0	0	0	0	1	1
_	4r79.2					Ш				$\vdash$	
_	K02E10.1	0	0	0	0	1	0	0	1	0	0
_	F11A5.4	0	0	0	0	0	0	0	0	0	1
_	F11A5.3 C56E6.2	-	-	-	-	-	-	-	-	U	
arl-1	F54C9.10	0	0	0	0	0	0	0	0	1	1
arf-1.2	B0336.2	0	0	0	0	0	0	0	0	1	1
arl-5	ZK632.8	0	0	0	0	0	0	0	0	1	1
arf-3	F57H12.1	0	0	0	0	0	0	0	0	1	1
arf-6	Y116A8C.12	0	0	0	0	0	0	0	0	1	1
arf-1.1	F45E4.1	0	1	0	0	0	0	0	1	0	0
evl-20	F22B5.1	0	0	0	0	0	0	0	0	1	1
arl-8	Y57G11C.13	0	0	0	0	0	0	0	0	1	1
arl-6	C38D4.8	0	0	0	0	0	0	0	0	1	1
arl-3	F19H8.3	0	0	0	0	0	0	0	0	1	1
_	ZK180.4	1	0	0	0	0	0	0	0	1	1
_	Y54E10Br.2	0	0	0	0	0	0	0	0	1	1
-	Y37E3.5	0	0	0	0	1	0	0	1	0	0
arc-1	ZK1320.6	4	_		_		_	_			0
ran-1	K01G5.4	0	0	0	0	0	0	0	0	0	0
let-60 ras-1	ZK792.6 F17C8.4	0	0	0	0	1	0	0	1	0	0
ras-2	C44C11.1	0	0	0	0	0	0	1	1	0	0
Drn-1	C54A12.4	Ť	H	H	Ť	H	٣	_	_	H	_
rap-1	C27B7.8	0	0	0	0	1	0	0	1	0	0
rap-2	C25D7.7	0	0	0	0	1	0	0	1	0	0
rap-3	C08F8.7	Ť	Ť		Ť		Ť	Ť		Ť	
rheb-1	F54C8.5	0	0	0	0	0	0	0	0	1	1
ral-1	Y53G8Ar.3	0	0	0	0	1	0	0	1	0	0
rho-1	Y51H4A.3	0	0	0	0	0	0	0	0	1	1
rac2	K03D3.10	0	0	0	0	0	0	0	0	1	1
cdc42	r07G3.1	0	0	0	0	0	0	0	1	0	0
crp-1	Y32F6B.3	0	0	0	0	1	0	0	1	0	0
		_									_
mig-2	C35C5.4						$\perp$				
chw-1	C35C5.4 F22E12.2										
	C35C5.4										

**Table S3**: Tissue expression was assessed using GTPase promoter::GFP fusions or data were retrieved for the *C. elegans* Gene Expression Consortium.

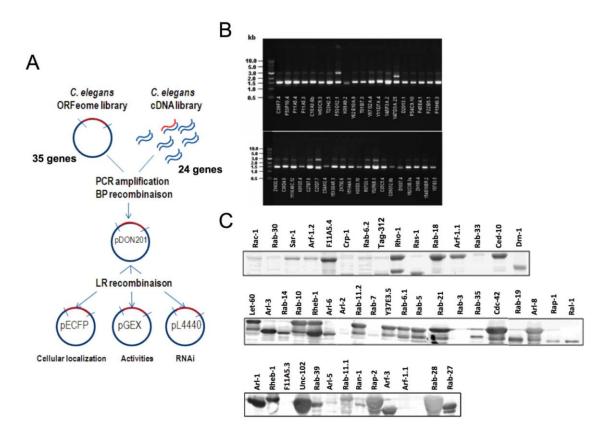
C elegans General design	nation																							Tis	ssu	lar I	oca	lizat	ion	1																					
gene name (Worm	Base)	$\overline{}$	_	$\overline{}$	$\overline{}$	$\overline{}$	$\overline{}$	$\overline{}$	$\neg$	$\neg$	$\overline{}$	$\overline{}$	$\overline{}$	$\overline{}$	$\overline{}$	$\neg$	$\overline{}$	$\neg$	_			_	_	_		_	Т	_	_	_	$\overline{}$	$\overline{}$	_	_	_				_	_		_	_	$\overline{}$	$\overline{}$	_	_	_		$\overline{}$	-
		amphid socket cells	amphids		anal sphincter	Arcade cells	body neurons	body wall muscle	coelomocytes	developing gonad	developing spermateca	developing uterus	Developing vuiva		dorsal nerve ring	mechanosensory neurons	excretory cell	excretory gland cells	gonad sheath cells	head mesodermal cell	head neurons	hypodermis	intestinal	intestinal muscle	lateral nerve cords/commissures	migrating hypodermal cell	nerve ring	oviduct sheath cell		Pharvagal paircoss	Pharyngeal-intestinal valve	Pharvnx	phasmids	rectal epithelium	rectal gland cells	reproductive system	seam cells	sheath cells	spermatheca	spermatheca-uterine valva	tail neurons	unidentified body	unidentified cells	unidentified head	unidentified tail	uterine muscle	uterine-seam cell		ventral nerve cord	vulva muscle	vulva other
rab-1 C39	F7.4	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0		1	0	1	1	0		1 C		0 (	0 0	0 1	1 0	0		0		0	0	0	1	0	0			(	) (	0 0		1	0
rab-2/unc-108 F53F rab-3 C18/		0				0	0	0	0	0	0			0	0	0	0	0	0			0	1					0 0		0 0		0 (	1 0									0						0 0		0	0
rab-5 F261		0			0	0	o	0	0	0	0			0	0	o	0	0	0	0												0 0																0 0			0
rab-6.1 F59E		0		0	0	0	0	0	0	0	0			0	0	0	0	0	0	0			0					0 0				0 (										0	0					0 0			0
rab-6.2 T250 rab7 W03		0	0	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0		0	0		0			1 0				0 0										0			0 0			0 0	0	0	0
rab7 W030 rab-8 D100		0			0	0	0	0	0	0	0			0	0	0	0	0	0	0												0 0													1 6			0 0			0
rab-10 T23E	H2.5	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1		1 0		1 (	0 (	0 (	0	0	0	1	0	1	0	0	(	0	0	) (		0	) (	0 0	0	0	
rab11.1 F53G		0			0	0	0	0	1	0	0		0	0	0	0	0	0	1	0			0		0						0 (	0 (		0	0					0	(							0 0			0
rab14 K09/ rab-18a Y92C		0			0	0	0	0	0	0	0			0	0	0	0	0	0	0			0		0							0 0			0					0								0 0			0
rab-19 Y62E		0			0	0	0	0	0	0	0			0	0	0	0	0	0	0			0									0 0			0		0			0								0 0		0	0
rab-21 T011	B7.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		0 (	0 0	0 (	0	0	0	0	0	0	0	0	(	0	0		) (	) (	) (	0 0		0	0
Aex-6 Y87G		0			0	0	0	0	0	0	0		0	0	1	0	0	0	0	0		0	1	0	0							0 0			0		0	0		0								0 0	1	0	0
rab-28 Y11D rab-30 Y45F	07A.4	0			0	0	0	0	0	0	0	0		0	0	0	0	0	0	0		0	0				1	1 0			0 0	0 (	1 0		0	0	0	0		0	(	0						0 0	0	0	0
rab-33 F43I	D9.2	0			0	0	0	0	0	- 61	0			ö	히	0	0	0	0	0		0	0		10		1					0 0			0	0	0	0	10	0	(			1 6				0 0	0	0	0
rab-35 Y47D3		0			0	0	0	0	0	0	0			0	0	0	0	0	0	0		0	1	0	0	0	1		1			0 0			0		0	0	0	0								0 0	0	0	0
rab-37 W011		0			0	0	0	0	0	0	0			0	0	0	0	0	0	0		0	0									0 (			0		0	0										0 0		0	0
rab-39 D20 rabY1/tag312 C33D		0			0	0	0	0	0	0	0			0	0	0	0	0	0	0		0	0	0							0 (	0 1	1 0		0		0	0		0	(		0					0 0	0	0	0
rabY1/tag312 C33D - 4r7		0			0	0	0	0	0	9	0			0	허	0	0	0	0	0			10				+					0 0			10	0	8	0	10	0								0 0	0	0	0
- K02E		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0		0	0	1				0 0	0 0			0	0	0	0		0					) (	) (	) (	0 0	0	0	0
- F112		0			0	0	0	0	0	0	0			0	0	0	0	0	0	0			0									0 (				0	0	0										0 0	0	0	0
- F11/		0			0	0	0	0	0	0	0			0	0	0	0	0	0	0		0	0				1					0 0			0	0	0	0	0	0	(							0 0	0	0	0
arl-1 F54C		0			0		0	0	0	7	0			6	허	ö	0	0	0	8		0	1	0				1 0							10	0	6	6	1 6	0	1	d						0 0	1	0	0
arf-1.2 B033	36.2	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	0	1	1	1	1	1	0	1	1 0		0 (	0 (	0 1	1 0	1	0	0	1	0	1	0	1	O		) (	) (	) (	) (	0 0	1	0	1
arl-5 ZK6		0			0		0		0	0	0			0	0	0	0	0	0	0				0	0							0 (					0											0 0		0	0
arf-3 F57H arf-6 Y116A		0			0		0	1	0	0	0			0	0	0	0	0	0	0				0	0		1					0 (	1 0				0	0										0 0		1	0
arf-1.1 F451		0					0	0	0	0	0			0	0	0	0	0	0	0												0 0																0 0		0	
evl-20 F221		0	0	0	0		0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0		0	0	1	-	0 0		0 (	0 0	0 (	0	0	0	0	0	1	1	0	(	0	0		) (	0	) (	0 1	0	0	0
arl-8 Y57G1		0			0	0	1	1	0	0	0			0	0	0	0	0	0	0		0	1	0								0 1	1 0				0			0		0						0 0		1	0
arl-6 C381 arl-3 F198		0					0	0	0	0	0			0	0	0	0	0	0	0	0	0										0 0					0				(	0						0 0		0	0
- ZK1			0	0	0		ŏ	0	0	0	0			0	0	ŏ	0	0	0	0	0									0 (	0 0	0 0									(							0 0		0	0
- Y54E1	10Br.2	0	0	이	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0		0 (	0 0	0 1	1 (	1	0	0	0	0	0	0	(	0	0		) (	) (	) (	0 0	0	0	1
- Y371		0					0	0	0	0	0			0	0	1	0	0	0	0		0						1 0				0 (		0			0			0		0			9			0 0		0	0
arc-1 ZK13 ran-1 K010		0					0	0	0	0	0			0	0	0	0	0	0	0												0 0																0 0		0	0
let-60 ZK7		0					0	1	0	ŏ	0			0	0	ŏ	0	0	0	0			1	0								0 1	1 0							0								0 0		0	1
ras-1 F170	C8.4	0	0	0	0		0		0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0		0 0		0	1 (	0 1	1 (	0	0	0	0	0	0	0		C	0		) (	) (	) (	0 0		1	0
ras-2 C440		0					0	0	0	0	0			0	0	0	0	0	0	1	0											0 (					0			0								0 0		0	0
rap-1 C271	A12.4 B7.8	0					0	0	0	0	0			0	0	0	0	0	0	0		0	1	0				1 0				0 0				0	0			0		0						0 0		0	0
rap-2 C25I		0					0	0	0	히	0			0	0	0	1	0	0	0		0	1	0	0			1 0				0 1	1 0			0	0			0		1	1 0					0 1	1	1	0
rap-3 C081	F8.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0 0		0 (	0 0	0 (	) (	0	0	0	0	0	0	0	(		0		) (	) (	) (	0 0		0	0
rheb-1 F540		0					0	0	0	0	0			0	0	0	0	0	0	0												0 0					0			0								0 0		0	0
ral-1 Y53G rho-1 Y51H		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0		0 0		1	1 (	0 (	) (	0	0	0	0	0		0	(	0 0	1	) (	) 1	1 0	1	0 0	0	0	1
rac2 K03E		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			1 0		0 (	0 0	0 0	0	0	0		0			0	(	0	0	0	0	0	) (	0 0	0	0	0
ede42 r070	G3.1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0		1 C	)	1	1 '	1 1	1 1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1 1	1	1	1
crp-1 Y32F		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0					0 (	0 (	0 (	) (	0	0					0	(	0	0		) (	) (	) (	0 0	0	0	0
mig-2 C356 chw-1 F22E		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0		1 0		0 (	0 (	0 (	0 0	0 0	0	0		0		0	(	0 0	1	) (	) (	) (	1	0 0	0	0	0
ced-10 C09G							1		0	9				1	0	ö	0	0	0				1									0 7											1 0					0 0		0	0
rab-11.2 W046									0	0				0	0	0	0	0	0				0					0					0															0 0			0
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**Table S4:** *C. elegans* small **G** proteins RNAi phenotypes. RNAi phenotypes were either collected from the worm RNAi database (red) or generated experimentally using a feeding protocol as previously described (31). Positive observation (1); negative observation (0).

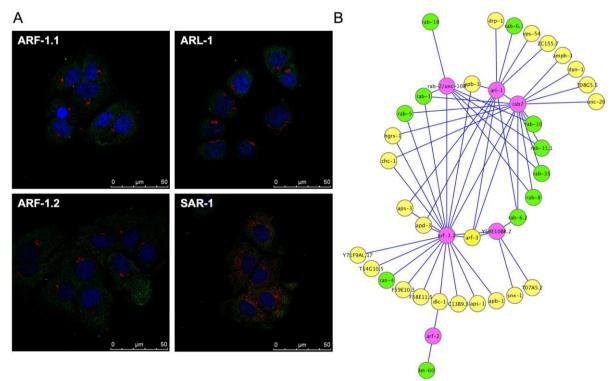
C elegans gene name	Gene designation (WormBase)																						R	NAi F	heno	type																						
		П	Т	Τ	Π				П	Т	Т	Т	Τ	T	Τ	Τ	Τ	Τ	Τ	Т	T	Т	Τ			Π	Π						Т	Τ	Τ	Τ	Τ	Τ	Τ	Π	Γ	Τ	Τ				Г	
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						<u>@</u>						amb (EMB)		(B)				(CAAD)	(EWD					early								(8)						2										
						e (EMB)					(F)	dwa		up (EV				dway	à aili					abnormal								P (EM						(EME	ial ear	_								
		8				que lle				(awa)	GE .	- la andre a		arly en			-	Hun north	0.00													rly em		CHAR			orma	ly emt	phorm	norms								
		sesduoo			EMB)	four cell			(9)	emb (EMB)	l early	C L		rmale			mount	inone		(d)			rmal	organelles								inct ea		(CITIE)			nent abno	nal ear	ance	tion at								
		le cell	()	â	) quie	normal	(CED)		Mal (N	early	mou.	A GILLS		/ apno	F		le jue	0.00	annee di	A) BVIL			n adnorr	5				le le	II UNC			indist			norma			moude	appear	caliza	_						2	
		Jermiji	IA) ler		rearly	cell contacts abno	ormal		apnor	plex phenotype early	ics at	Da ea		nearit	aldE	1 .	, london	photo osmodic pressure	2	ugh vr	D/G	e lou	firation	mbrar	(A)	est	E	ic leth	norma		٥	ncleus	6	phologo	per ad	9	o dev	sition	clear 8	Inlank	Va (P	d size		3RO)			y (STP)	
		lated	ariy let	(BLI)	le slow	conta	ith abn	CLR)	ration	bhen '	dynan	pi cien	(DPY)	notic ir	le leth	in an	olo de	0	Outro I	od thro		ent ab	loro lle	of me	rest (L	val arr	T) leu	mbrior	gon ab	(NC	ernal sterile	al pron	defect (MLT)	BAI W	mnue	PB PB	novuqu	dle po	ear nu	subce	luy gu	d brook	(SCK)	) ųjamo	(Slu)		поделу	
		accumulated germline cell	adult early lethal (ADL)	blistered (BLI)	cell cycle slow early emb (EMB)	lla cell	oell death abnormal (CED)	Clear (CLR)	oell migration abnormal (MIG)	omple	control dynamics abnormal early emb	and and a	dumpy (DPY)	egg osmotic integrity abnormal early emb (EMB)	embrionic lethal (LET)	combination of particular	embryonic development abnormal	orbein	or load	exploded through vulva (RUP)		fat content abnormal	germ cell prolifiration	ntegrity of membranous	larva arrest (LVA)	Late larval arrest	larva lethal (LET)	larval/embrionic lethal	locomotion abnormal	(NOT) Buol	atem	maternal pronucleus indistinct early emb (EMB)	molt defect	mus vuiva	neurone number adnormal	paralyzed (PRL)	post embryonic develops	P0 spindle position abnormal early emb (EMB)	pronuclear nuclear appearance abnormal early	protein subcellular localization abnormal	protruding vulva (PvI)	reduced brood size	sick (SC	slow growth (GRO)	sluggish	llems	sterile p	E
Rab-1	C39F7.4	0	1 (	0 0	0	0		0		0	0	0 (	) 1	1 0	1	0	) (	) (	0	0 (	0		) (		1	0	1	0			1 0	0	0	0 (		1	0	0	0	0	0		0	1		0	0	0
rab-2/unc-108 Rab-3	F53F10.4 C18A3.6	0	0 '	1 0	0	0	0	0	0	0	0	0 (	) (	) (	1	0 1	1 (	1 (	n	0 1	<u>1</u>	0 0	1	0	0	0	0	0	0	0	0	0	0	n r	1 0	1 0	10	n	n	0	10	0	0	0	1	0	0	0
rab-5 Rab-6.1	F26H9.6 F59B2.7	0	0 (	0 0	0	0	0	0	0	0	0	0 (		0 0		1 0			0	0 0	0	0 0	0 0	0	0	0	0	0	0 0	0	0	0	0	0 0			0	0	0	0	0	0	0	0	0	0	0	0
Rab-6.2	T25G12.4 W03C9.3	0	0 (	0 0	0	0	0	0	0	0	0	0 0	) (	) (	1 (	n T	0 0	) (	0	0 0	0	0 (	) (	0	0	0	0	0	0	0	0	0	0	0 0	1 0	) (	- 0	10	0	0	10	10	0	0	0	0	0	0
Rab-8 Rab-10	D1037.4 T23H2.5	0	0 (	0 0	0	0	0	0 0	0	0 0	0	0 (	0 0	0 0	1	1 1			0	0 1	0	0 (	0 0	0	0	0	0	0	0 0	0	1	0	0	0 0	0 0	0	0	0	0	0	0 0	0	0	0	0	0	0	0
Rab11.1	F53G12.1	0	0 (	0 0	0	0	0	0	0	0	0	D I (	) [ (	) I 1		1			0	0 (	0	0 (	0	0	1	0	1	0	0	0	1	0	0	0 0		0	0	0	0	0	0	0	1.0	0	0	0	0	0
Rab14 Rab-18a	K09A9.2 Y92C3B.3	0	0 (	0	0	0	0	0 0	0	0	0 0	0 (	) (	) (		0 0		) (	0	0 (	0	0 0	) (	0	0 0	0	0	0	0 0	0	0 0	0	0	0 (	0 0		0 0	0	0	0	0	0	0	0	0	0	0	0
Rab-19 Rab-21	Y62E10A.9 T01B7.3	0	0 (	0 0	0	0	0	0 0	0	0	0	0 (	) (	0 0	) (	1 1		) (	0	0 1	0	0 0		0	0 0	0	0 0	0	0	0	0	0	0	0 0	0 0	) (	0	0	0	0	0 0	0	0	0	0	0	0	0
Aex-6 Rab-28	Y87G2A.4 Y11D7A.4	0	0   0	)   0		0	0	0	0	0	0	0 (	) (	0 0		0 0	0 0	) (	0	0 (	0	0 (	0 0	0	1	0	0	0	0	0	0	0	0	0 (	0 0	) (	0	0	0	0	1	0	0	0	0	1	0	0
Rab-30 Rab-33	Y45F3A.2 F43D9.2	0	0 (	0 0	0	0	0	0	0	0 0	0	0 (		0 0		0 0		0 (	0	0 (	0	0 0	0 0	0	0	0	0	0	0 0	0	0	0	0 0	0 0	0 0	0	0	0	0	0	0 0	0	0	0	0	0	0	1
Rab-35	Y47D3A.25	0	0 (	0 0	0	0	0	0	0	0	0	0 (			Ì	0 0			0	0 (	0	0 0	0	0	0	0	0	0	0	0	0	0	0 0	0 0	0	Ö	0 0	0	10	0				0	0	0	0	1
Rab-37 Rab-39	W01H2.3 D2013.1	0	0 (	0 0	0	0	0	0	0	0	0 0	0 (		) (		0		1	0	0 (	0	0 (	0 0	0	0	0	0	0	0	0	0	0	0	0 0	) (		0	0	0	0	10	10	0	0				1
RabY1/tag312	C33D12.6 4R79.2	0	0 (	0 0	0	0	0	0 0	0	0	0 0	0 (		0 0		0 0		0 (	0	0 (	0	0 0	0 0	0	0	0	0	0	0 0	0	0 0	0	0	0 0			0 0	0	0	0	0	0		0	0	0	0	1
-	K02E10.1 F11A5.4	0		0 0	0	0	I 0 I	0 1	0	0	0	0 (	) (	0   0	)   (	ווס	)   (	7	0   1	0   0	ן כ	1   1	0 0	0	0	0	0	0	0	0	0	0	0	0   0	) I (	) I (	10	10	0	0	10	10	0	0	0	0	0	0
-	F11A5.3 C56E6.2	0	0 (	0 0	0	0	0	0	0	0	0	0 (	) (	0	0	0 0	0 0	) (	0	0 (	0	1 1		0	0	0	0	0	0	0	0	0	0	0 0	0 0	) (	0	0	0	0	0	0	0	0	0	0	0	0
arl-1 arf-1.2	F54C9.10 B0336.2	0	0 (	0 0	0	0	0	0	0	0	0	0 (	) (	0 0	1	1 1		) (	0	0 0	0	0 (	) (	0	0	0	0	0	0	0	0	0	0	0 0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
arl-5	ZK632.8	0	0 (	0 0	0	0	0	0	0	0	0	0 (				1	1 (		0	0 0	0	0 0	1	0	0	0	0	0	0	0	1	0	0	0 (	) (	0	0 0	0	0	10	0	0	0	0	0	0	0	0
arf-3 arf-6	F57H12.1 Y116A8C.12	0	0 (	0	0	0	0	0	0	0	0	0 (	) (	0	1	1 0	) [(	) [ (	0   1	0 1	5	0 (	0 0	0	0	0	0	0	0	0	0	0	0	0 0			0	0	0	0	0	0	0	0	0	0	0	0
arf-1.1 evi-20	F45E4.1 F22B5.1	0	0 (	0 0	0	0	0	0	0	1	0	0 (	) (	0	1	1   1	) 1		0	0 (	0	0 0	) (	0	0	0	0	0	1	0	1	0	0	0 (	0 0		0	0	0	0	1	0	0	1	0	0	1	0
arl-8 arl-6	Y57G11C.13 C38D4.8	0	0 (	0	0	0	I 0 I	0	0	0 0	0 0	0 (	) (	0	1	0 0		) ( ) (	0	0 0	0	0 0	0 0	0	0	0	0	0	0	0	0	0	0	0 0			0 0	0	0	0	0 0	0	0	0	0	0	0	0
arl-3	F19H8.3 ZK180.4	0	0 (	0 0	0	0	0	0	0	0	0	0 (		0 0	) (	0 0	0 0	) (	0	0 1	0	0 0	0 0	0	1 1	0	0	0	0	0	0	0	0	0 0	0 0	0 0	0	0	0	0	0	0	0	0	0	0	0	0
-	Y54E10BR.2 Y37E3.5	0	0 (	0 (	0	0	0	0	0	0		0 (		0 0		0 0		) (	0	0 (	0	0 (	0 0	0	0	0	0	0	0	0	0 0	0	0	0 0		0	0 0		0	0	0	0	0	0	0	0	0	1
arc-1	ZK1320.6 K01G5.4	0	0 (	0	0	0	0	0	0	0	ŏ	0 0		0		0		) I (	0	ŏ	0	ŏ	0	0	10	0	0	10	0	0 1	0	0	0	0 0		j	Ó	0	0	0	10	10		0	0	0	0	1
ran-1 Let-60	ZK792.6	0	0 1	1 0	0	0	0	0		0	0	0 (	1	0		1		) (	0	0 1	0	0 0	0 0	0	1	1 1	1	0	1	0	1	0	0	0 0			0 0	0	0	0	0	0	0	0	0	0	0	0
Ras-1 Ras-2	F17C8.4 C44C11.1	0	0 (	0 0	0	0	0	0	0	0	0	0 (	) (	) (	1	0			0	0 (	0	0 0	0 0	0	0	0	0	0	0	0	0	0	0 0	0 (	) (		0 0	0	0	0	0	0	0	0	0	0	0	0
Drn-1 Rap-1	C54A12.4 C27B7.8	0	0 (	0 0	0	0	0	0	0	0	0	0 0	0 0	0 0		ווס	0   0	) (	0	0 0	0	0 0	0 0	0	0	0	0	0	0	0	0	0	0	0 0	0 0	0 0	0	0	0	0	10	10	10	0	0		0	1
Rap-2	C25D7.7 C08F8.7	0	0 (		0	0	0	0	0	0 0	0	0 (	0 0	0	1	0 1		1	0	0 1	2	0 0		0	0	0	0	0	0 0 0	0	0	0 0	0	0 (		0	0 0	0	0	0	0	0	0	0	0	0	0	
Rap-3 Rheb-1	F54C8.5	0	0 (	0 0	0	0	0	1	0	0	0	0 (		0		0 1	) 1		0	0 (	5	ŏ	0	0	0	0	0	0	1	1	0	0	0	0 0		0	1 1	1 0	0	0	0	0	0	1	0		1	0
Ral-1 Rho-1	Y53G8AR.3 Y51H4A.3	0		0 0	0	0	0	0		0	1	0 (	) (	0	1	1	1 0	) ( ) (	0	0 1	0	0 0	0	0	0	0	0 1 0	0	1	0	1 1 0	0	0	0 (	0 1	0	0 0	0	0	0	0 0	0	0	0	0	0	0	0
rac2 Cdc42	K03D3.10 R07G3.1	0	0 (	) 1	0	0	0	0	1	0	0	0 (	) (	0 0	)   (	ווח			0	0 0	0	0 0		0	1	0	0	0	0	0	1	0	0	1 1	1 0		0	0	0	0	1	0	0	10	0	0	1	0
crp-1 Mig-2	Y32F6B.3 C35C5.4	0	0 (	0 0	0	0	0	0	0	0	0	0 (	) (	0 0	) (	0 0	0 0	) (	0	0 0	0	0 0	0 0	0	0 0	0	0	0	0	0	0	0	0	0 0	0 0	) (	0	0	0	0	0 0	0	0	0	0	0	0	1
Chw-1 Ced-10	F22E12.2 C09G12.8	0	0 (	0 0	0	0	0	0	0	0	0	0 (		0 0	Ì	0			0	0 0	2	0 0	0 0	0	0	0	0	0	0	0	0	0	0 0	0 0			0	0	0	0	0	0	Ó	0	0	0	0	1
Rab-11.2	W04G5.2	0	0 0	0	0	0	0	0	0	0	0	0 0		0	1	1		1	0	0	0	0 0	0	0	1	0	1	0	0	0	1	0	0	0 0		0	0	0	0	0	0	0	0	1	0	0	0	0

**Table S5: GTPase co-expression data.** Co-expression (1); no co-expression or information not documented (0 or blank).

C elegans gene name	Gene designation (WormBase)																												c	:o-	exp	res	sic	n (	(mF	RN.	A)																										
		C09G12.8a	K03D3.10	R07G3.1	C36C6.4	2303.4	Y32F6B.3	Y51H4A.3	C39F7.4	F53F10.4	C18A3.6b	W03C9.3	T23H2.5	F53G12.1	K09A9.2	Y62E10A.9	T01B7.3	V87G2A 4	V44D7A 4	Y45F3A.2	24300 3	V47D3A 26	200404	2013.1	F11A5.4	F11A5.3	F54C9.10	C38D4.8	ZK632.8	F19H8.3	Y116A8C.12	F45E4.1	F22B5.1	K01G5.4	C27B7.8	C25D7.7	CE4A42.4	Y53G8AR.3	747026	N/92.6	FZ6H9.6	13982.1	125612.4	W04G5 2	Y92C3B 3	W01H2.3	C33D12 6	4P79.2	K02E10 1		2000	B0336.2	Y57G11C.13	ZK180.4	Y54E10BR.2	Y37E3.5	ZK1320.6	C44C11.1a	F17C8.4	F54C8.5	C08F8.7	FZZE1ZZ	F57H12.1
Rab-1	C39F7.4	0				1	0		1	0		>				7				0 (			0 6		0	-	-	0	2 0	1	٠	0	0	×	0				0	1 1		0		0 >		0 0			0			0	0	0	0	0	0	0	-	0			0
rab-2/unc-10		0			_	0	0		0	1	0	_				_				0 0	0		-	~	0	0	1	0	1	0	0	0	·	0			_	,	_	0		-	_	-		0 0		_	0		0	1	0	0	1	0	1	0	0	0			0
Rab-3	C18A3.6	0			0	0	1	0	0	0	1	C				0			1	1 (	0				0	0	0	0	0	0	0	0	0	0				1 (	0	0	0	0	0	0		0 .	_	0	1				0	0	0	0	0	0	1	0		0	0
rab-5	F26H9.6	0			~	1	0		1	0	<del>-</del>	· ·				(				0 (	~		-		0	0	0	0	0	1	0	0	0	0			0	0 (	0	1	_	0	-	0		0 (		-	0			9	0	0	0	0	0	0	0	0	0		0
Rab-6.1	F59B2.7	0				0	0		0	0			0 0			0				0 (					0	0	0	0	0	0	0	0	0	0				0 (	0	0	0	0		0		0 0			0			0	0	0	0	0	0	0	0	0			0
Rab-6.2	T25G12.4 W03C9.3	0				0	0		0	1	0		1							0 (	-				0	0	1	0	1	0	0	0		0		Н		0 (		0		_				0 0			_		0	1	0	0	0	0	1	0	0	0			0
Rab-8	D1037.4	0				0	0		0	0															0	0	0	0	0	0	0	0						0					0			0 0						0	-	0	0	0	0		0	0			0
Rab-10	T23H2.5	0	(	1	0	0	0	0	0	0	0	0	) '	1		0	) (		0	0 4	1	0	0	0	0	0	0	0	0	0	0	0	0	1	C		0	0 (	0	0	0	0	0	0		0 0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rab11.1	F53G12.1		L	I	T	_	$\Box$					$\perp$	I	$\perp$	$\perp$	$\perp$	I	T	$\perp$	$\perp$	Ŧ	$\perp$	Ţ	1	4	$\Box$	_		$\Box$						$\perp$	I	Ŧ	$\perp$	T	_	_	1	_	$\perp$	I	$\perp$	I	T	$\perp$	I	4	$\perp$	4	$\dashv$	$\Box$	_	_	$\Box$	_	$\dashv$	$\perp$	_	_
Rab14	K09A9.2	0	0	1	0	1	0	0	1	0	0	0	1	) (	1	(	) (	4	0	0 (	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	0	0 (	0	1	1	0	1	0	1	0 0		1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0
Rab-18a Rab-19	Y92C3B.3 Y62E10A.9	0	-	١,	+		0	0	0	0	0	0	1	0	1	1	١,	1	0	0 0	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	+	+		1	0	0	0	0	0	+	0 0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rab-21	T01B7.3	0				0	0		0	1	0	_				-			ŏ						0	0	1	0	1	0	0	0	-	0				0 0								0 0		_			0			0	1	0	1	0	0	0			0
Aex-6	Y87G2A.4	0				0	1	0	0	0		C							1						0	0	0	0	0	0	0	0	0	0	0		0									0		D					0	0	0	0	0	0	1	0			0
Rab-28	Y11D7A.4	0				0	1	0	0	0		C							1	1 (					0	0	0	0	0	0	0	0	0				0									0 ,		0						0	0	0	0	0	1	0			0
Rab-30	Y45F3A.2	0				0	0		0					1 (						D ,	_				0	0	0	0	0	0	0	0			C											0 (								0	0	0	0		0	0			0
Rab-33	F43D9.2	0	0	1	9	0	0	0	0	1	0	1	1	0	1	1	) '	4	0	0 (	0	1	0	0	0	0	1	0	1	0	0	0	1	0	1	-	0	0 (	0	0	0	0	0	0	1	0 (	4	D .	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0
Rab-35 Rab-37	Y47D3A.25 W01H2.3	0	-	1		0	1	0	0	0	1	0	1	0	1	1	0	1	1	1 (	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	1 (	0	0	0	0	0	0	,	0 .	٠	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Rab-37	D2013.1	0				0	0		0										0	0 0			0		0	0	0	0	0	0	0	1	0													0 0							0	1	0	0	0		0	0			0
RabY1/tag31		0				1	0		1	0						(				0 0					0	0	0	0	0	1	0	0	0	0			0	0 (	0	1	1	0	_	0		0 0		1	0			0	0	0	0	0	0	1	0	0	0		0
-	4R79.2	0	0	1	0	0	1	0	0	0	1	C	0	0	) (	0	0	)	1	1 (	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	1 (	0	0	0	0	0	0		0 .	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
-	K02E10.1	0	0			1	0		1	0		0		_		(			0		0			0	0	0	0	0	0	1	0	0	0	0	0		0	0 (	0	1	1	0	_	0	1 1		)	1	0	_	0	0	0	0	0	0	0	1	0	0	0		0
_	F11A5.4	1	_1	1		0	0		0	0						9				0 0	0				0	1	0	0	0	0	0	0	0				0					0		0					0			0	0	0	0	0	0	0	0	0			0
_	F11A5.3 C56E6.2	0		0		0	0		0	0			0 0			_	-			0 (			-		0	0	0	0	0	0	0	0	0	0	+		0	_				0				0 0		-		0		0	0	0	0	0	0	0	0	0			0
arl-1	F54C9.10	0				0	0		0	1	0		1			1 6	,	-	0		n	-	0		0	0	1	0	1	0	0	0	1	0	1		<del>}</del>	0 0			0	0	0	0 1	1	-		1	-	~	0	1	0	0	1	0	1	0	0	0			0
arf-1.2	B0336.2	0				0	0		0	1	0		(			0	,			0 (	0		-		0	0	1	0	1	0	0	0	1	0	1			0 0		0	0	0	0	0	0			0			0	1	0	0	1	0	1	0	0	0			0
arl-5	ZK632.8	0			0	0	0		0	1	0		(							0 (	0				0	0	1	0	1	0	0	0	1	0	1			0 0	0	0	0	0				0 0					0	1	0	0	1	0	1	0	0	0			0
arf-3	F57H12.1	0		) (	0	0	0		0	0	0	_				0				0 (	0				0	0	0	0	0	0	0	0	0	0				0 (			0	0	0					0				0	0	0	0	0	0	0	0	0		0	1
arf-6	Y116A8C.12	0		1		0	0		0	0		0				-	0			0 (	0				0	1	0	1	0	0	1	0	0	0			0	0 (	-	0	0	0			1			0	0			0	0	0	0	0	0	0	0	0			0
arf-1.1	F45E4.1 F22B5.1	0				0	0		0	0	0	0	0			0 0	) (	_	0	0 0	0		0		0	0	0	0	0	0	0	1	0	0	0		0	0 (		0	0	0							0		0	0	0	0	0	0	0	0	0	0			0
evl-20 arl-8	Y57G11C.13	0				0	0		0	0						1 0	,			0 0	n				0	0	0	0	0	0	0	0	0	0	0		-	-				-				0 0		-				0	1	0	0	0	0	0	0	1			0
arl-6	C38D4.8	0				0	0		0	0										0 0					0	1	0	1	0	0	1	0	0	0	-		ŏ	0 (				-				0 0		-	-			0	0	0	ő	0	0	0	0	0			0
arl-3	F19H8.3	0		0	0	1	0		1	0	0	0	0			(	0			0 (	0				0	0	0	0	0	1	0	0	0	0	0		0	0 (	0	1	1	0	1	0	1			1	0			0	0	0	0	0	0	1	0	0			0
-	ZK180.4	0				0	0		0	0										0 (			0		0	0	0	0	0	0	0	0		0												0 (						0	0	1	0	0	0	0	0	0			0
-	Y54E10BR.2	0	0	1	0	0	0	0	0	1	0	1	(	) (	) (	0	) ,	1	0	0 (	0	1	0	0	0	0	1	0	1	0	0	0	1	0	1		0	0 (	0	0	0	0	0	0		0 (		0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0
	Y37E3.5		H,	+	+				_	١.	١.	١.	۲.	١,	١,	٠,	1	+	+	١,		+	+	_		_	_		_	_	_	_	٠,	_	١.	H	+	٠.	+	_	1	_	_	1	+	١.	+	+	-		,		0				-		_	_	1	1	
arc-1	ZK1320.6 K01G5.4	0				0	0		0	0	0			0			0 0			0 0	1				0	0	1	0	0	0	0	0	0	1	C		0		0	0		0		0 1		0 0		0	0		0	0	0	0	0	0	0	0	0	0			0
Let-60	ZK792.6	0				1	0		1	0						0				0 0	_				0	0	0	0	0	1	0	0	0	0	0			0 0	_	1		0	-	0	1			-	0			0	0	0	0	0	0	1	0	0	0		0
Ras-1	F17C8.4	0				0	1	0	0	0		c							1	1 (					0	0	0	0	0	0	0	0	0				0	1 (		0						0		0	1				0	0	0	0	0	0	1	0			0
Ras-2	C44C11.1	0			0	1	0		1	0	0	0	) (			(	) (		0	0 (	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0		_	0 (	0	1	1	0	1	0	1	0 (	)	1	0	1		-	0	0	0	0	0	1	0	0	0		0
Dm-1	C54A12.4	0				0	1	0	0	0	-	C				-	_	-	1	1 (	οŢ		0	0	0	0	0	0	0	0	0	0	0	0	0	-	0	_				0		0			1	PΓ	1			0	0	0	0	0	0	0	1	0			0
Rap-1	C27B7.8	0				0	0		0	1	0		(			-				0 (	_				0	0	1	0	1	0	0	0	1	0			0	0 (				0	0			0 (					0	1	0	0	1	0	1	0	0	0			0
Rap-2	C25D7.7 C08F8.7	0				0	0		0	0										0 0					0	0	0	0	0	0	0	0	0		-		+	0 (				0	0			0 0		-				0	0	0	0	0	0	0	0	0			0
Rap-3 Rheb-1	F54C8.5	0				0	0		0	0										0 0					0	0	0	0	0	0	0	0	0					0 (								0 0						0	1	0	0	0	0	0	0	1			0
Ral-1	Y53G8AR.3	0				ō	0		0	0										0 0	-				0	0	0	0	0	0	0	0	0	0				0			-	0	0		5								0	0	0	0	0	0	0	0			0
Rho-1	Y51H4A.3	$\perp$		İ	I	⇉				L		I	I	I	I	I	I	İ	I	I	I	I	I	╛	⇉	J									I	İ	I	1	1			I			I	I	I	I	I	I		⇉	╛	J		╛		J	J	J	⇉		
rac2	K03D3.10	1	1		_	0	0		0											0 (					0	1	0	1	0	0	1	0	0	0			-	0 (					-			0 (							0	0	0	0	0	0	0	0			0
Cde42	R07G3.1	0				0	0		0			_							0	0 (	0			0	0	0	0	0	0	0	0	0	0	0				0 (					0			0 (							0	0	0	0	0	0	0	0			0
crp-1 Mig-2	Y32F6B.3 C35C5.4	0			_	0	0	0	0	0		0		_		0	_	_	0	1 (	0		0	0	0	0	0	0	0	0	0	0	0	0	0		0	0 (		0		0	-	0		0 0	_	0	0			0	0	0	0	0	0	0	0	0	0		0
Chw-1	F22E12.2	0				1	0		1	0						(									0	0	0	0	0	1	0	0							0	1		0		0		0 0			0					0	0	0	0	1	0	0			0
Ced-10	C09G12.8	1	1	-	_	0	0		0										0						0	1	0	0	0	0	0	0	0					-								0 0								0	0	0	0	0	0	0			0
rab-11.1	W04G5.2	0	0			1	0	0	1	0	0	0				(			0		0		0		0	0	0	0	0	1	0	0	0	0	0		0	0 (	0	1		0		0		0 (		1	0			0	0	0	0	0	0	1	0	0	0	1	0



**Figure S1: A**) Cloning strategies for GTPase ORF retrieval. 35 ORFs were retrieved from the C. elegans ORFeome and 24 were cloned using PCR amplification from *C. elegans* total cDNA. All the amplified PCR products were recombined into the pDONR201 vector and sequenced. **B**) Typical PCR product analysis of 40 GTPase encoding ORFs.**C**) Coomassie blue stained SDS polyacrylamide gel showing the expression level of *C. elegans* GST-tagged small G proteins expressed in bacteria and purified on glutathione-sepharose.



**Figure S2: A)** C-terminally FLAG-tagged small G proteins encoding plasmids were transfected into Vero cells and the cellular localization was revealed using anti-FLAG antibodies and immunofluorescence and confocal microscopy (red), an endoplasmic reticulum marker (calnexin) was also used (green). Nuclei were stained with DAPI. **B)** Membrane trafficking network comprising gpIII GTPases (see Figure 4 and the integration of new subcellular localization data) and its genetic and physical interactions as available in Wormbase. The network is similar to that obtained in Figure 4 (pink: gpIII GTPases).