

# Supplemental Materials

*Molecular Biology of the Cell*

Lindsay et al.

**Movie 1.** Myosin Va knockdown results induces the formation of GFP-Rab6A' positive tubules. A HeLa cell line stably expressing GFP-Rab6A' was transfected with control or myosin Va siRNAs for 72 hours and observed by spinning disk confocal microscopy. A z stack of seven planes was acquired every 2 s for a total duration of 2 minutes.

**Movie 2.** Myosin Va knockdown results in clustering of GFP-Rab11A-positive vesicles. HeLa cells transfected with control or myosin Va siRNAs for 72 hours and transiently expressing GFP-Rab11A observed by spinning disk confocal microscopy. A z stack of seven planes was acquired every 2 s for a total duration of 2 minutes.

**Movie 3.** Myosin Va knockdown results in clustering of GFP-Rab14-positive vesicles. HeLa cells transfected with control or myosin Va siRNAs for 72 hours and transiently expressing GFP-Rab14 observed by spinning disk confocal microscopy. A z stack of seven planes was acquired every 2 s for a total duration of 2 minutes.

**Movie 4.** Myosin Va-positive vesicles lack directionally. A431 cells stably expressing GFP-MyoVaFL(D)<sub>WT</sub> were observed by laser scanning confocal microscopy. Frames were acquired every 4 s for a total duration of 400 s. 5 $\mu$ M ionomycin was added to the culture medium after 40 s.

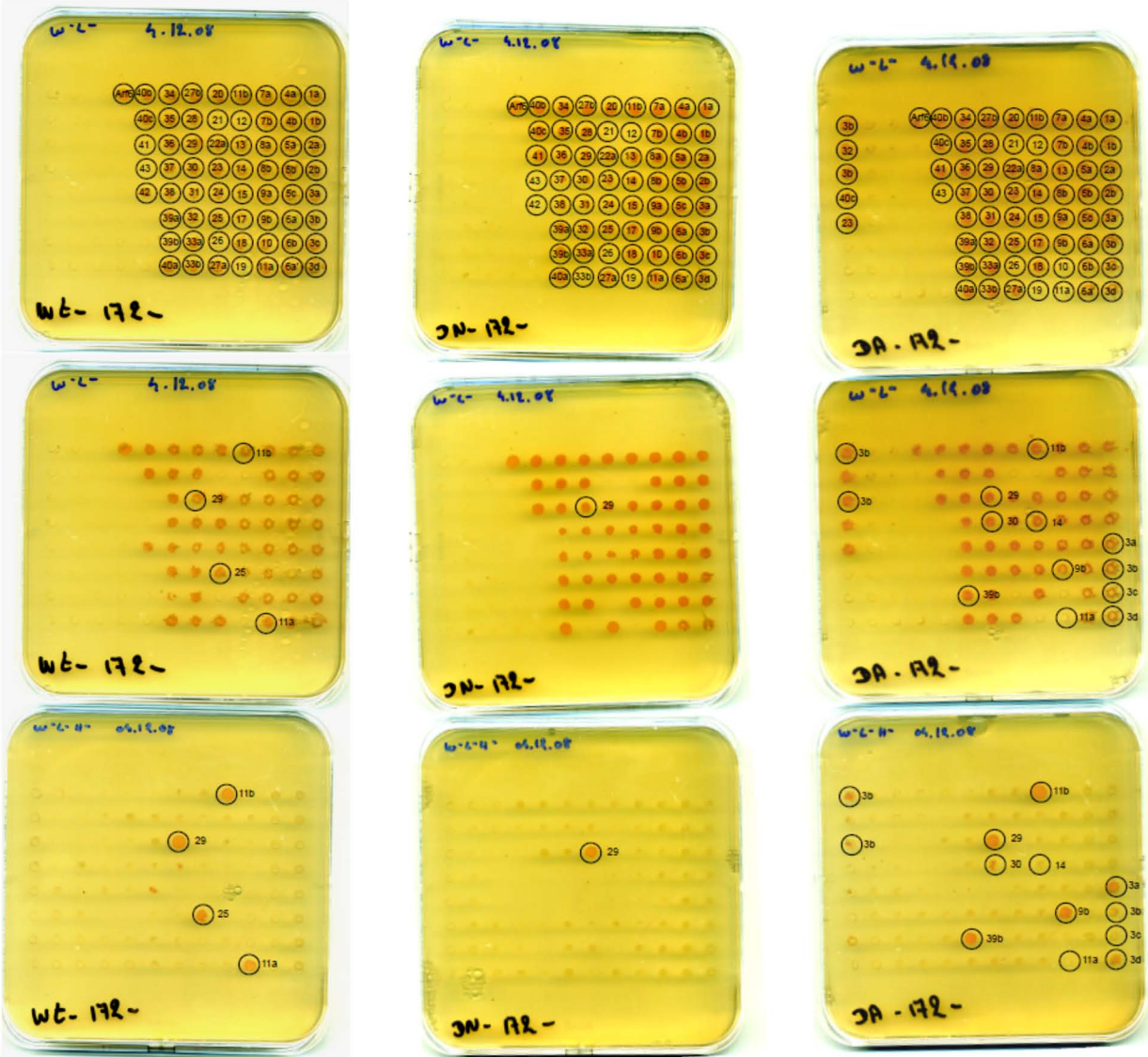
**Movie 5.** A constitutively-active mutant of myosin Va displays the same motility pattern as wild-type myosin Va. A431 cells expressing GFP-MyoVaFL(D)<sub>D136A</sub> were observed by laser scanning confocal microscopy. Frames were acquired every 4 s for a total duration of 240 s. (Note: ionomycin was not used).

**Supplemental Table S1.** Systematic yeast two-hybrid screen of myosin Va. Results from a high throughput yeast two-hybrid 'living chip' assay using pGADGH MyoVaT(F) as bait. Green boxes indicate a positive interaction (+ weak; ++ strong; +++ very strong) and orange boxes indicate no interaction. WT, wild-type; DA, dominant-active; DN, dominant-negative.

**Supplemental Table S2.** A list of the Rab DA and DN mutants used in the Y2H screen, and the primers used to generate the mutations.

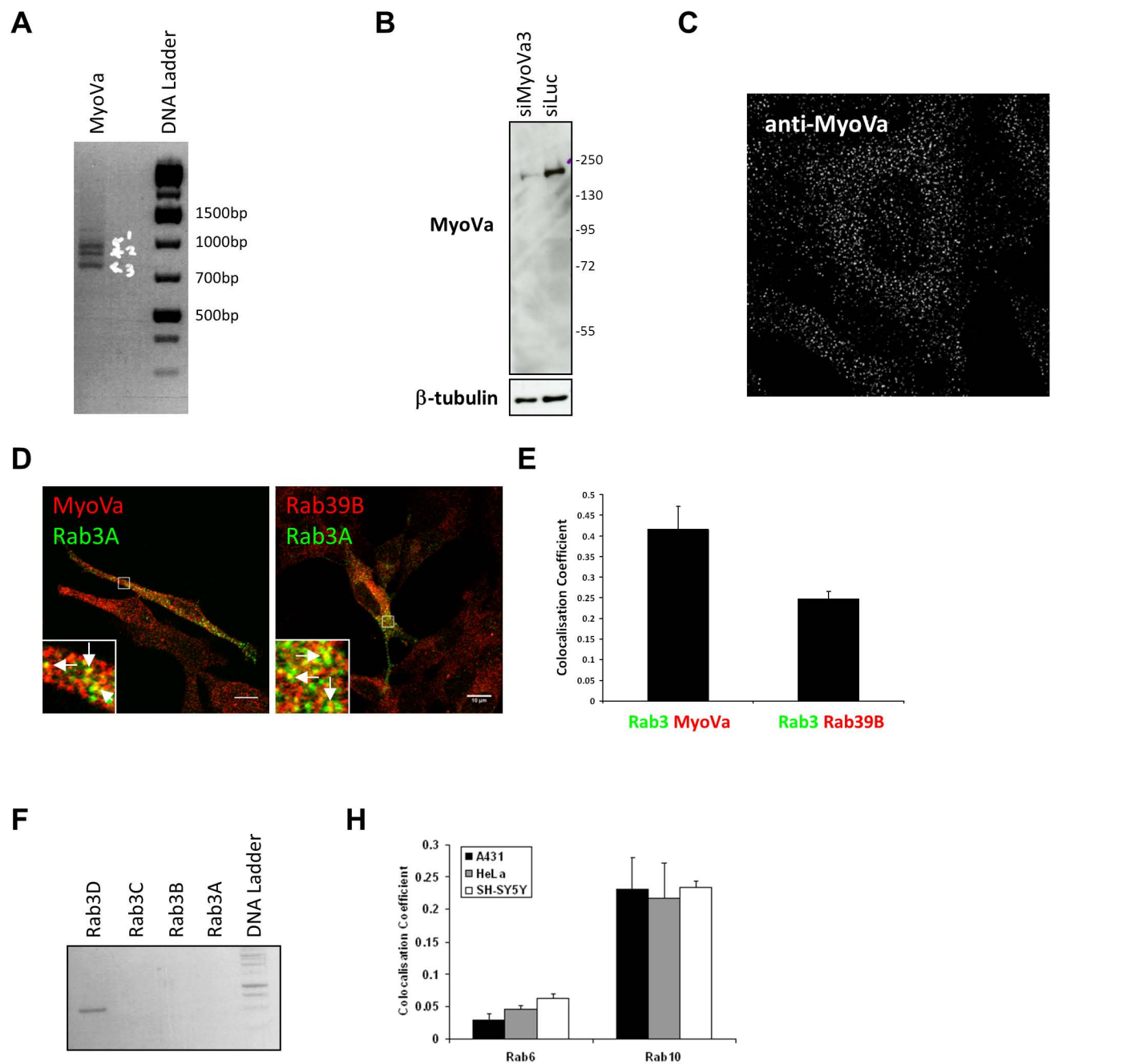
**Supplemental Table S3.** A list of the siRNA sequences used in this study.

**FIGURE S1**



**Figure S1.** Systematic yeast two-hybrid screen of myosin Va isoforms. Raw data of the yeast two-hybrid ‘living chip’ assay. *Top Row:* Histidine-containing plates with yeast colonies co-transformed with MyoVa(F)<sub>1100-1852</sub> and the indicated Rab GTPase. Encircled numbers refer to Rabs. WT – wild-type, DN – Dominant-negative, DA – Dominant-active. *Middle Row:* Replica of the plates in the top row grown on histidine-containing media. *Bottom Row:* Replica of the plates in the top row grown on histidine-lacking media. Colonies that grow in the absence of histidine contain interacting fusion proteins.

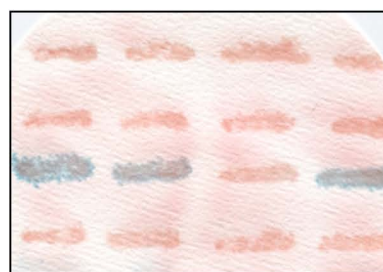
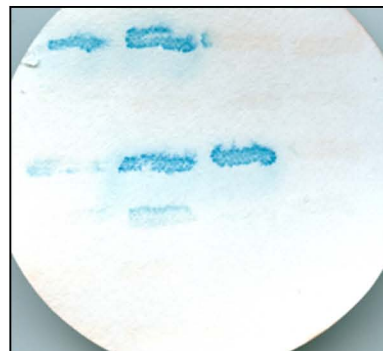
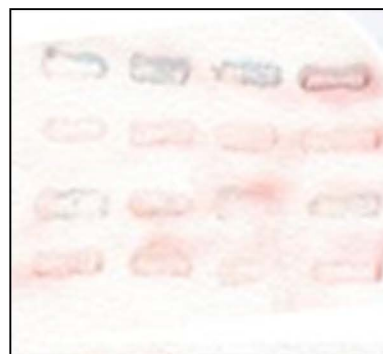
# FIGURE S2



**Figure S2.** (A) RT-PCR performed with HeLa RNA and primers flanking the alternatively spliced region of human myosin Va. Bands were cloned into the pGEM T Easy vector and sequenced. Band 1 contained fragments of MyoVaABCDE (D isoform) and MyoVaABCEF (F isoform), band 2 was MyoVaABCE and band 3 was from an unrelated mRNA transcript. (B) Extracts from HeLa cells transfected for 72 hours with control or myosin Va siRNA were separated by SDS-PAGE and analysed by western blot with anti-myosin Va.  $\beta$ -tubulin was used as a loading control. (C) A HeLa cell fixed and labelled with anti-myosin Va antibody. Shown is a deconvolved image of a single section taken through the equator of the cell. (D) SH-SY5Y cells fixed and labelled with antibodies to detect endogenous Rab3A (green) and myosin Va (red) or Rab39B (red). Bar, 10 $\mu$ m. (E) Quantitative analysis of the level of colocalisation between Rab3A and myosin Va or Rab39B (mean  $\pm$  s.e.m.  $n = 15 - 20$  cells). (F) RT-PCR performed with HeLa RNA and primers specific for each of the four Rab3 isoforms. Only Rab3D mRNA is expressed in the HeLa cells used in this study. (G) Quantitative colocalisation of endogenous myosin Va with endogenous Rab6 and Rab10 in the indicated cell lines.

**FIGURE S3**

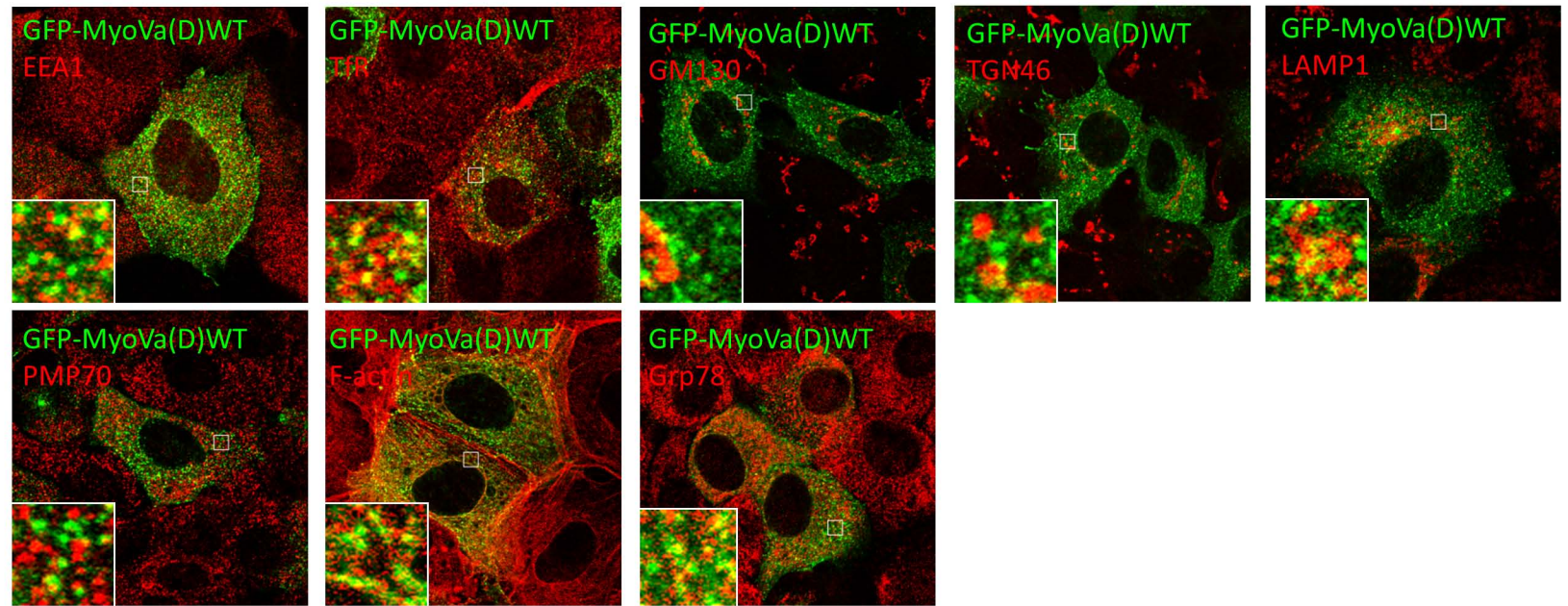
<u>Prey</u>	<u>Bait</u>
MyoVaT(F)	Rab3A.DA
MyoVaT(F)	Rab3A.DN
MyoVaT(F)	Rab3B.DA
MyoVaT(F)	Rab3B.DN
MyoVaT(F)	Rab6A.DA
MyoVaT(F)	Rab6A.DN
MyoVaT(F)	Rab6B.DA
MyoVaT(F)	Rab6B.DN
MyoVaT(F)	Rab6C.DA
MyoVaT(F)	Rab6C.DN
MyoVaT(F)	Rab9B.DA
MyoVaT(F)	Rab9B.DN
MyoVaT(F)	Rab11A.DA
MyoVaT(F)	Rab11A.DN
MyoVaT(F)	Rab11B.DA
MyoVaT(F)	Rab11B.DN
MyoVaT(F)	Rab25.WT
MyoVaT(F)	Rab25.DN
MyoVaT(F)	Rab14.DA
MyoVaT(F)	Rab14.DN
MyoVaT(F)	Rab39A.DA
MyoVaT(F)	Rab39A.DN
MyoVaT(F)	Rab39B.DA
MyoVaT(F)	Rab39B.DN



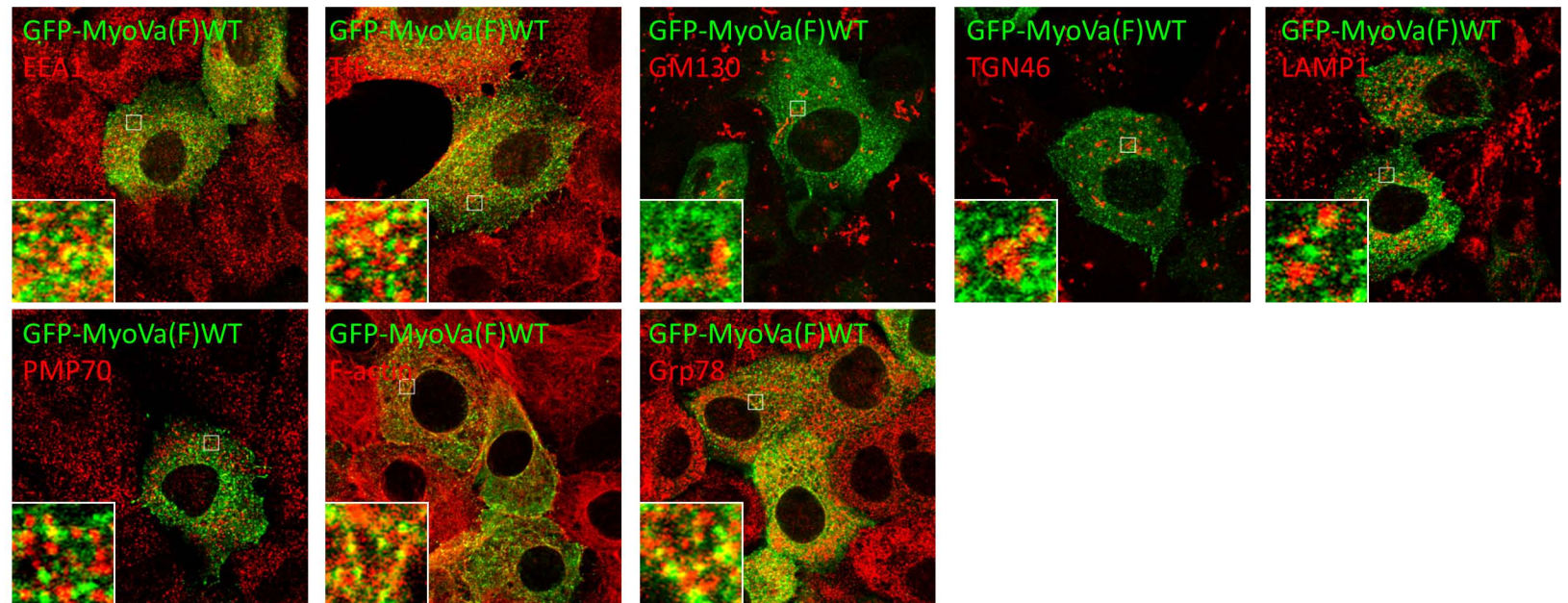
**Figure S3.** Secondary yeast two-hybrid screen of the interactions identified in the ‘living chip’ assay. Yeast patches transformed with the indicated bait and prey constructs were replica plated onto filter paper placed on agar dissolved in selection medium. After overnight incubation at 30°C the filters were removed, flash frozen in liquid nitrogen and placed on filters soaked in Z buffer containing X-gal and  $\beta$ -mercaptoethanol. A blue colour indicates an interaction between the bait and prey fusion constructs.

**FIGURE S4**

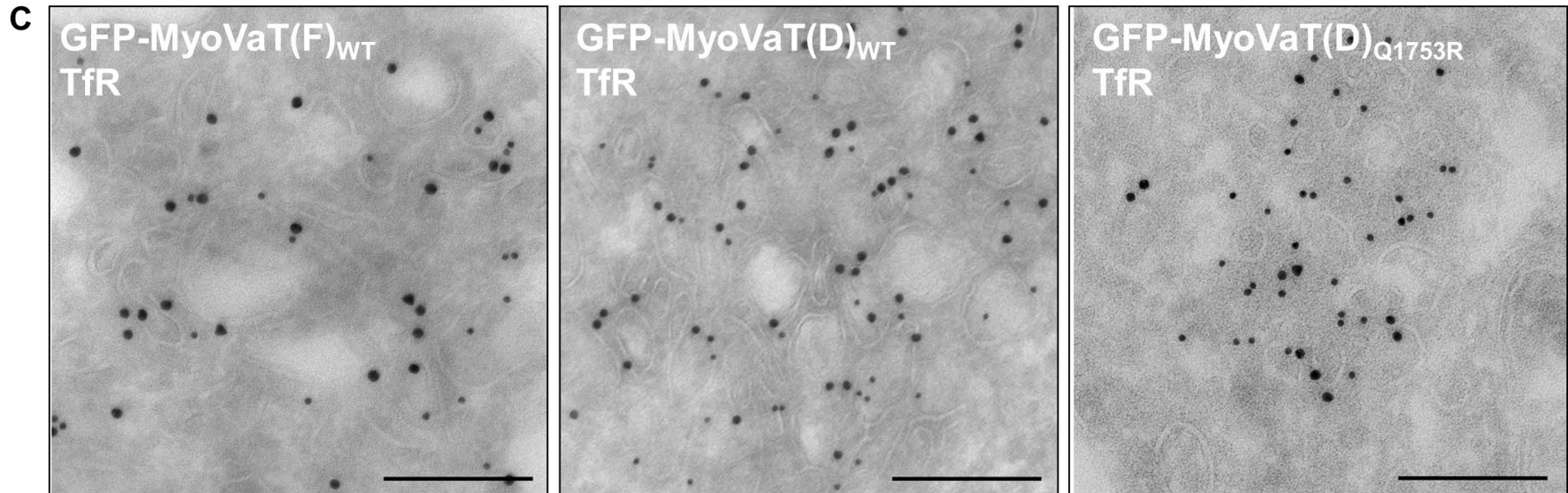
**A**



**B**



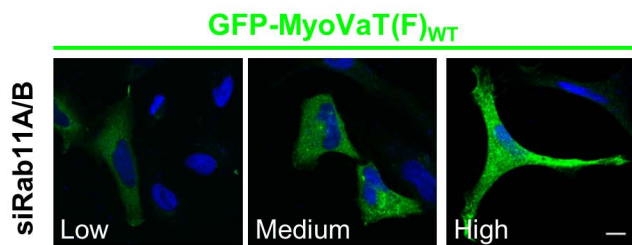
## FIGURE S4



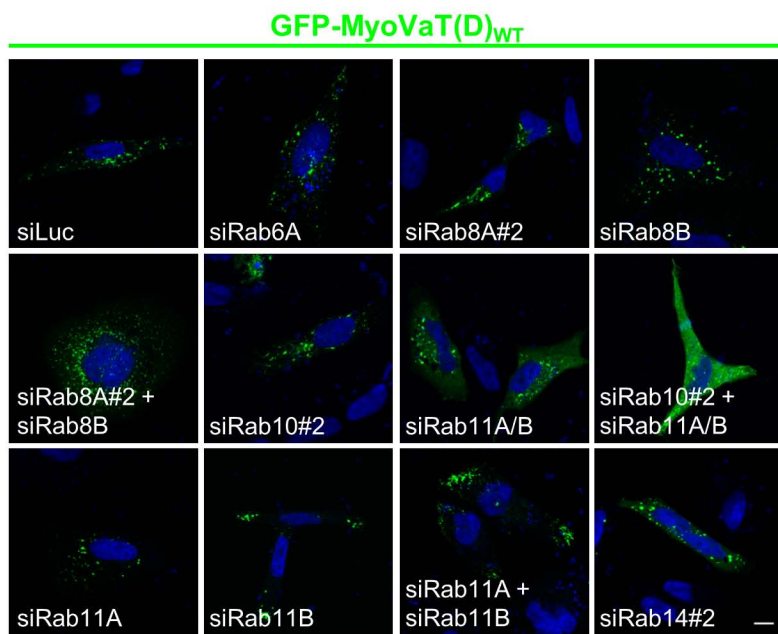
**Figure S4.** Myosin Va localises to compartments along the endocytic recycling pathway and the ER. (A) A431 cells expressing GFP-MyoVaFL(D)<sub>WT</sub> treated with 5 $\mu$ M ionomycin for 4 minutes prior to fixation and labelling with the indicated antibodies. (B) A431 cells expressing GFP-MyoVaFL(F)<sub>WT</sub> treated with 5 $\mu$ M ionomycin for 4 minutes prior to fixation and labelling with the indicated antibodies. (C) Electron microscopy images of HeLa cells expressing the indicated GFP-fused myosin Va tail construct processed for ultrathin cryosectioning, and double immunogold labelled with anti-GFP (15nm) and anti-TfR (10nm). Arrows indicate membrane-bound vesicles. Bar, 200nm.

# FIGURE S5

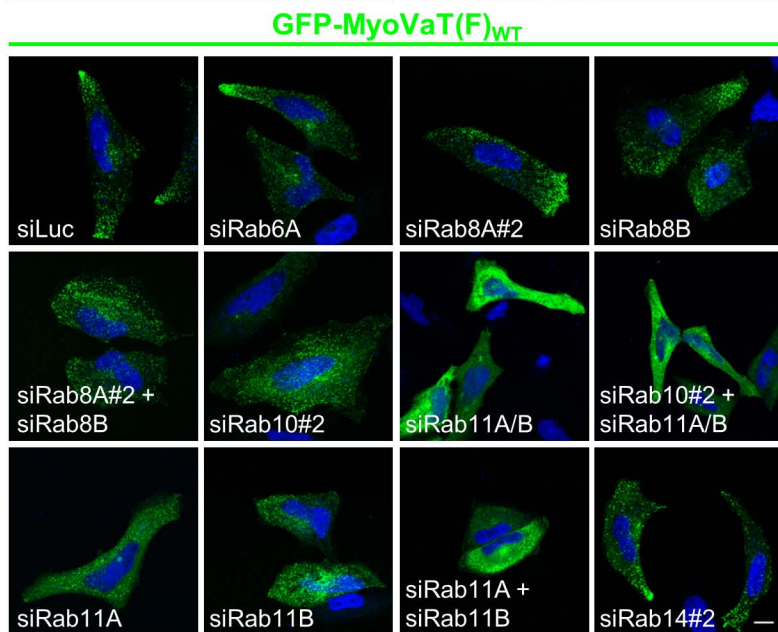
**A**



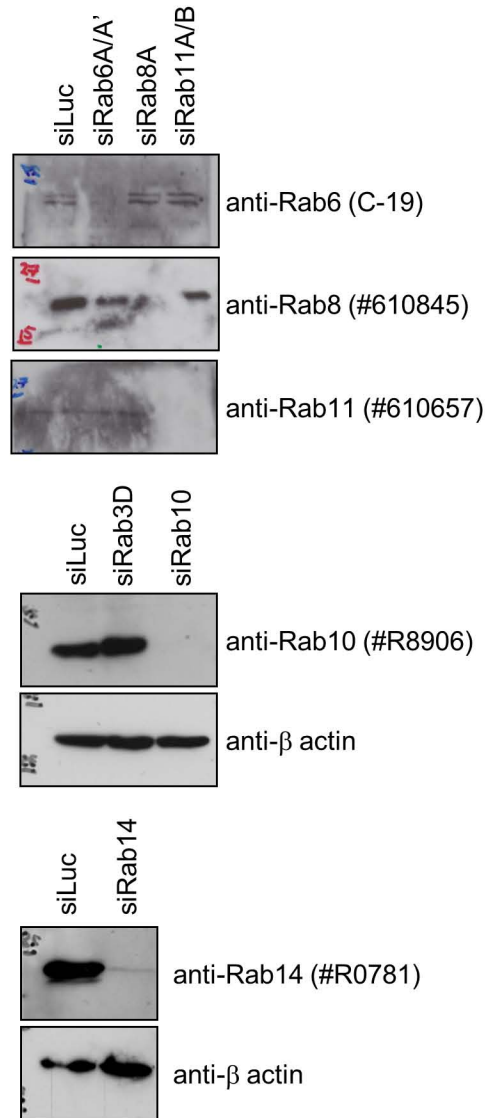
**B**



**C**



**D**



**E**

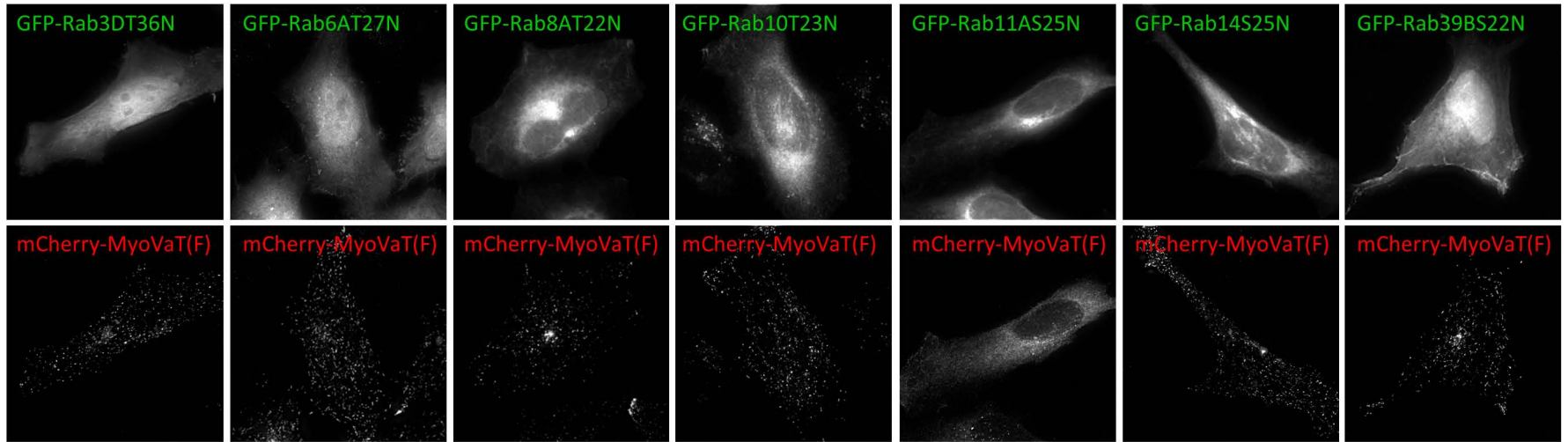
siRab6A	5' - GAGCGGUUCAGGAGCUUGA - 3'
siRab8A#2	5' - GACAAGUUCCAAGGAACG - 3'
siRab8B	5' - CAGGAAAGAUUCCGAACAA - 3'
siRab10#2	5' - AGGGUAAUGCAGAAGUGAU - 3'
siRab11A	Dharmacon (#L-004726-005)
siRab11B	Dharmacon (#L-004727-005)
siRab14#2	5' - CCUGUUGUGUGGGUGGCAU - 3'

**Figure S5.** Effect of Rab knockdown on myosin Va vesicular localisation. (A - C) Single confocal sections of HeLa cells transfected with the indicated siRNA duplex for 72 hours and expressing GFP-MyoVaT(F)<sub>WT</sub> (A and C) or GFP-MyoVaT(D)<sub>WT</sub> (B). Nucleus labelled with DAPI. (A) Representative cells expressing low, medium, and high levels of GFP-MyoVaT(F)<sub>WT</sub>. All images were recorded with the same confocal microscope settings. Bar, 10µm. (D) Representative Western blots of HeLa cell lysates transfected in parallel with the indicated siRNA duplexes for the experiment shown in Fig. 3 C - E. HeLa cells in 6-well dishes were transfected with siRNAs using HiPerFect transfection reagent and 48 hours post-transfection the cells were split into 24-well plates with or without glass coverslips. Twenty-four hours later (a total of 72 hours post-transfection) the cells in the wells without coverslips were lysed and analysed by Western blot. (E) Sequences of the siRNA duplexes used in B and C. See Table S1 for the sequences of siLuciferase and siRab11A/B.

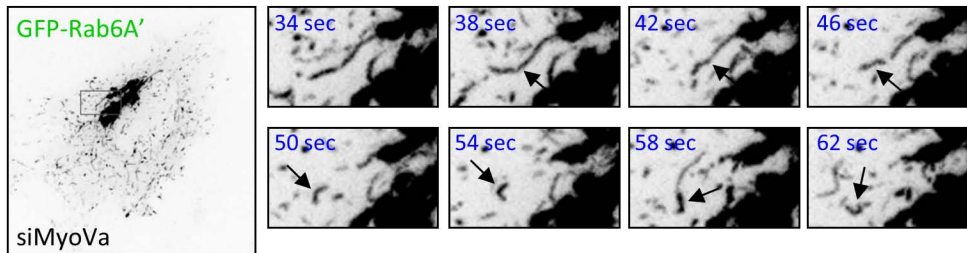


# FIGURE S6

A

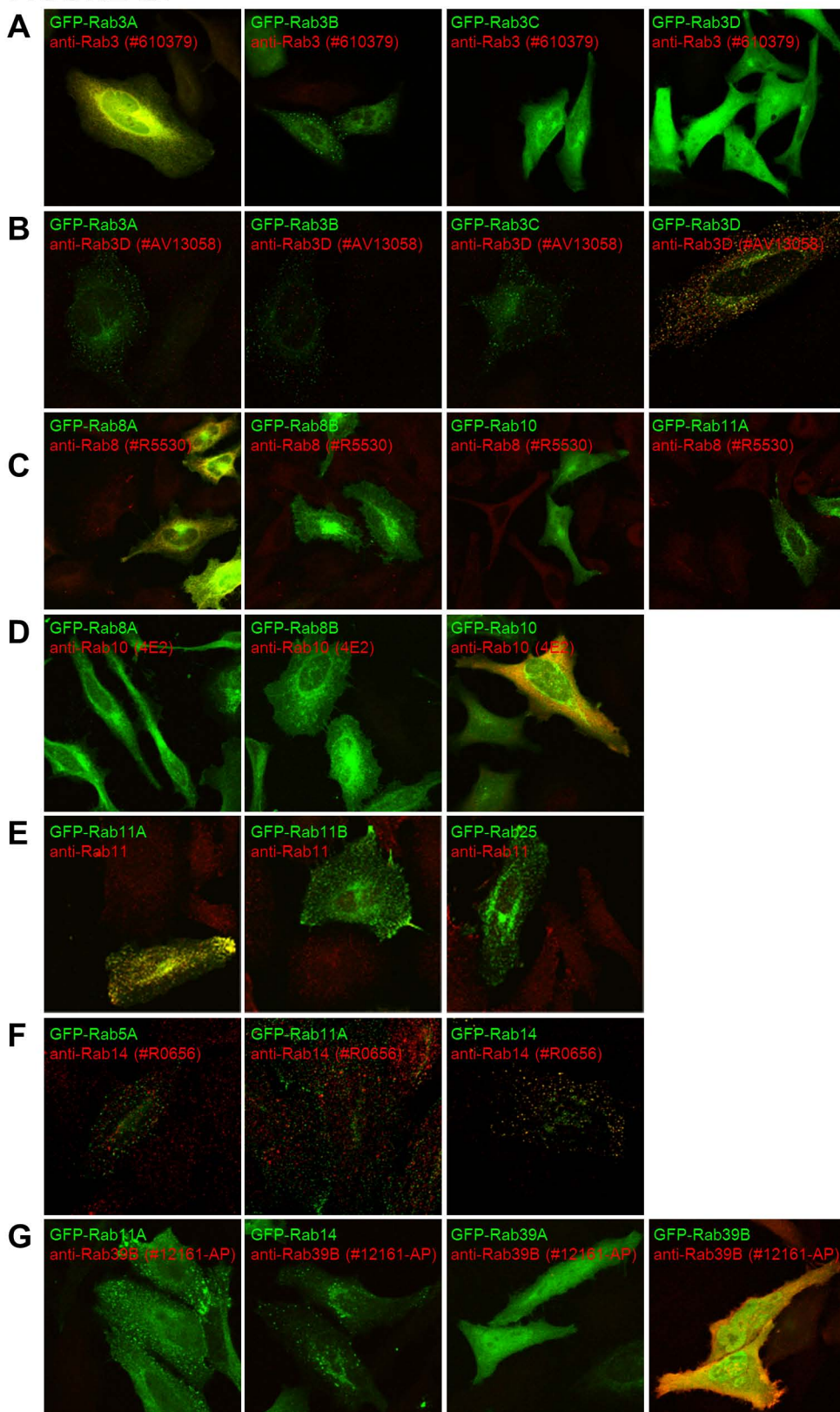


B



**Figure S6.** Dominant-negative Rab11 blocks myosin Va membrane binding. (A) HeLa cells were co-transfected with mCherry-MyoVaT(F)<sub>WT</sub> and GFP-fusions of the dominant-negative mutants of each interacting Rab GTPase. Transfected cells were fixed and processed for fluorescence microscopy. Shown are representative maximum intensity projections of deconvolved z stacks. (B) HeLa cells were transfected with control or myosin Va siRNA for 72 hours, and plasmid DNA encoding GFP-Rab6A' for the final 18 hours. A z-series was recorded at each time-point and shown are maximum intensity projections of a single representative time-point for each condition. The boxed region containing a tubule was magnified and frames at 4 second intervals are shown. Arrow indicates a transport carrier undergoing fission from the tubule. Shown are representative frames from Video 4.

# FIGURE S7



**Figure S7.** Characterisation of anti-Rab antibodies. HeLa cells were transfected with the indicated GFP-fused Rab GTPases (wild-type) and 24 hours later the cells were fixed and labelled with the indicated anti-Rab antibodies (red). Shown are overlay images. Each Rab antibody was tested against its target Rab and two or three of its closest relatives. (A) Mouse monoclonal anti-Rab3 (BD Biosciences) is specific for Rab3A. (B) Rabbit polyclonal anti-Rab3D (Sigma) is specific for Rab3D. (C) Rabbit polyclonal anti-Rab8 (Sigma) is specific for Rab8A. (D) Mouse monoclonal anti-Rab10 (AbCam) is specific for Rab10. (E) Rabbit polyclonal anti-Rab11 (homemade) is specific for Rab11A. (F) Rabbit polyclonal anti-Rab14 (Sigma) is specific for Rab14. (G) Rabbit polyclonal anti-Rab39B (ProteinTech) is specific for Rab39B.

**Supplemental Table S1**

HsRab1a	wt		HsRab6c	wt		HsRab19	wt		HsRab33b	wt	
	DA			DA			DA			DA	
	DN			DN			DN			DN	
HsRab1b	wt		HsRab7a	wt		HsRab20	wt		HsRab34	wt	
	DA			DA			DA			DA	
	DN			DN			DN			DN	
HsRab2a	wt		HsRab7b	wt		HsRab21	wt		HsRab35	wt	
	DA			DA			DA			DA	
	DN			DN			DN			DN	
HsRab2b	wt		HsRab8a	wt		HsRab22a	wt		HsRab36	wt	
	DA			DA			DA			DA	
	DN			DN			DN			DN	
HsRab3a	wt		HsRab8b	wt		HsRab23	wt		HsRab37	wt	
	DA	++		DA			DA			DA	
	DN			DN			DN			DN	
HsRab3b	wt		HsRab9a	wt		HsRab24	wt		HsRab38	wt	
	DA	+		DA			DA			DA	
	DN			DN			DN			DN	
HsRab3c	wt		HsRab9b	wt		HsRab25	wt	+	HsRab39a	wt	
	DA	+		DA	++		DA			DA	
	DN			DN			DN			DN	
HsRab3d	wt		HsRab10	wt		HsRab26	wt		HsRab39b	wt	
	DA	+		DA			DA			DA	++
	DN			DN			DN			DN	
HsRab4a	wt		HsRab11a	wt		HsRab27a	wt		HsRab40a	wt	
	DA			DA	+++		DA			DA	
	DN			DN			DN			DN	
HsRab4b	wt		HsRab11b	wt		HsRab27b	wt		HsRab40b	wt	
	DA			DA	+++		DA			DA	
	DN			DN			DN			DN	
HsRab5a	wt		HsRab12	wt		HsRab28	wt		HsRab40c	wt	
	DA			DA			DA			DA	
	DN			DN			DN			DN	
HsRab5b	wt		HsRab13	wt		HsRab29	wt	+++	HsRab41	wt	
	DA			DA			DA	+++		DA	
	DN			DN			DN	+++		DN	
HsRab5c	wt		HsRab14	wt		HsRab30	wt		HsRab42	wt	
	DA			DA	++		DA	+		DA	
	DN			DN			DN			DN	
HsRab6a	wt		HsRab15	wt		HsRab31	wt		HsRab43	wt	
	DA	++		DA			DA			DA	
	DN			DN			DN			DN	
Rab6A'	wt		HsRab17	wt		HsRab32	wt				
	DA			DA			DA		DA		
	DN			DN			DN		DN		
HsRab6b	wt		HsRab18	wt		HsRab33a	wt				
	DA	++		DA			DA		DA		
	DN			DN			DN		DN		

## Supplementary Table S2

<b>Rab Mutation</b>	<b>Primer Sequence (5'-3')</b>
HsRab1a_Q70L_sens	TGGGACACAGCAGGCCTAGAAAGATTTTCTGAACA
HsRab1b_Q67L_sens	TGGGACACAGCGGGCCTAGAACGGTTCGGGACC
HsRab2a_Q65L_sens	TGGGATACGGCAGGGCTAGAAATCCTTTTCGTTCC
HsRab2b_Q65L_sens	TGGGATACGGCTGGGCTAGAAATCCTTCCGTTCT
HsRab3a_Q81L_sens	TGGGACACAGCAGGGCTAGAGCGGTACCGGACC
HsRab3b_Q81L_sens	TGGGACACAGCTGGGCTAGAGCGGTACCGGACC
HsRab3c_Q89L_sens	TGGGACACAGCAGGCCTAGAAAGATACAGGACT
HsRab3d_Q81L_sens	TGGGACACAGCGGGCCTAGAGCGCTACCGCACC
HsRab4a_Q67L_sens	TGGGATACAGCAGGACTAGAACGATTCAGGTCC
HsRab4b_Q67L_sens	TGGGACACGGCTGGCCTAGAGCGGTTTCGGTCA
HsRab5a_Q79L_sens	TGGGATACAGCTGGTCTAGAACGATACCATAGC
HsRab5b_Q79L_sens	TGGGACACAGCTGGGCTAGAGCGATATCACAGC
HsRab5c_Q80L_sens	TGGGACACAGCTGGACTAGAGCGGTATCACAGC
HsRab6a_Q72L_sens	TGGGATACTGCGGTCTAGAACGTTTCCGTAGC
HsRab6b_Q72L_sens	TGGGACACAGCTGGTCTAGAGAGGTTCCGCAGC
HsRab6c_Q72L_sens	TGGGATACGGCAGGTCTAGAACGTCTCCGTAGC
HsRab7a_Q67L_sens	TGGGACACAGCAGGACTAGAACGGTTCAGTCT
HsRab7b_Q67L_sens	TGGGACACGGGCGGTCTAGAGCGGTTCCGCTCC
HsRab8a_Q67L_sens	TGGGACACAGCCGGTCTAGAACGGTTTCGGACG
HsRab8b_Q67L_sens	TGGGACACAGCGGGTCTAGAAAGATTCCGAACA
HsRab9a_Q66L_sens	TGGGACACGGCAGGTCTAGAGCGATTCCGAAGC
HsRab9b_Q66L_sens	TGGGACACTGCAGGGCTAGAACGTTTCAAGAGC
HsRab10_Q68L_sens	TGGGATACAGCAGGCCTAGAGCGATTTACACACC
HsRab11a_Q70L_sens	TGGGACACAGCAGGGCTAGAGCGATATCGAGCT
HsRab11b_Q70L_sens	TGGGACACCGCTGGCCTAGAGCGCTACCGCGCC
HsRab12_Q67L_sens	TTCACCGACGACACCCTATGCGAGGCCTGCAAG
HsRab13_Q67L_sens	TGGGACACGGCTGGCCTAGAGCGGTTCAAGACA
HsRab14_Q70L_sens	TGGGATACGGCAGGACTAGAGCGATTTAGGGCT
HsRab15_Q67L_sens	TGGGACACTGCAGGGCTAGAGAGATACCAGACC
HsRab17_Q77L_sens	TGGGACACAGCTGGCCTAGAGAAGTACCACAGC
HsRab18_Q67L_sens	TGGGATACTGCTGGTCTAGAGAGGTTTAGAACA
HsRab19_Q123L_sens	TGGGACACAGCTGGCCTAGAGCGCTTCCGCACC
HsRab20_R59L_sens	TGGGACACCGCAGGGCTAGAGCAGTTCACGGC
HsRab21_Q78L_sens	TGGGATACGGCAGGTCTAGAGAGATTCCATGCA
HsRab22a_Q64L_sens	TGGGATACAGCTGGACTAGAACGATTTTCGTGCC
HsRab23_Q68L_sens	TGGGACACTGCAGGTCTAGAGGAATTTGATGCA
HsRab24_S67L_sens	TGGGACACAGCAGGCCTAGAGCGCTATGAGGCC
HsRab25_L71Q_sens	TGGGACACAGCTGGCCAAGAGCGGTACCGAGCC
HsRab26_Q123L_sens	TGGGACACAGCTGGTCTAGAGCGGTTCCGCAGT
HsRab27a_Q78L_sens	TGGGACACAGCAGGGCTAGAGAGGTTTCGTAGC
HsRab27b_Q78L_sens	TGGGACACTGCAGGGACTAGAGCGGTTCCGGAGT
HsRab28_Q72L_sens	TGGGATATAGGAGGGCTAACAATAGGAGGCAA
HsRab29/Rab7L1_Q67L_sens	TGGGATATTGCAGGGCTAGAGCGCTTACCTCT
HsRab30_Q68L_sens	TGGGACACAGCAGGTCTAGAGAGATTTCCGTCC
HsRab31_Q65L_sens	TGGGACACTGCTGGTCTAGAACGGTTTCATTCA
HsRab32_Q85L_sens	TGGGACATCGCGGGGCTAGAGCGATTTGGCAAC
HsRab33a_Q95L_sens	TGGGACACAGCAGGTCTAGAACGTTTCCGCAA
HsRab33b_Q92L_sens	TGGGACACAGCAGGACTAGAACGATTCAGAAAG
HsRab34_Q111L_sens	TGGGATACCGCTGGGCTAGAGAGGTTCAAATGC
HsRab35_Q67L_sens	TGGGACACAGCGGGGCTAGAGCGCTTCCGCACC
HsRab36_Q182L_sens	TGGGACACAGCTGGGCTAGAGAAGTTCAAGTGC
HsRab37_Q89L_sens	TGGGACACCGCTGGGCTAGAACGGTTCGAAGC

HsRab38\_Q69L\_sens TGGGATATCGCAGGTCTAGAAAGATTTGGAAAC  
HsRab39a\_Q72L\_sens TGGGACACGGCGGGACTAGAGCGGTTTCAGATCA  
HsRab39b\_Q68L\_sens TGGGATACCGCGGGTCTAGAGAGGTTTCAGATCC  
HsRab40a\_Q73L\_sens TGGGATACGTCGGGGCTAGGAAGATTTTGTACC  
HsRab40b\_Q73L\_sens TGGGATACTTCAGGCCTAGGAAGATTTTGTACC  
HsRab40c\_Q73L\_sens TGGGACACGTCGGGCCTAGGCCGGTTCTGCACC  
HsRab41\_Q89L\_sens TGGGACACAGCTGGCCTAGAGCGCTTTCACAGC  
rab42\_QL\_sens TGGGACACGGCCGGCCTAGAGCGGTTCCGCACC

HsRab1a\_S25N\_sens TCAGGGGTTGGAAAGAACTGCCTTCTTCTTAGG  
HsRab1b\_S22N\_sens TCAGGCGTGGGCAAGAAGAACTGCCTGCTCCTGCGG  
HsRab2a\_S20N\_sens ACAGGTGTTGGTAAAAACTGCTTATTGCTACAG  
HsRab2b\_S20N\_sens ACAGGTGTGGGGAAGAAGAACTGTCTCCTCCTGCAG  
HsRab3a\_T36N\_sens AGCAGCGTGGGCAAGAAGAACTCCTTCCTCCTCCGC  
HsRab3b\_T36N\_sens AGCAGTGTGGCAAGAAGAACTCCTTCCTCCTCCGC  
HsRab3c\_T44N\_sens AGCAGTGTGGGGAAGAAACTCTTTTCTATTCCGT  
HsRab3d\_T36N\_sens AGCAGTGTGGGCAAGAAGAACTCCTTCCTGTTCCGA  
HsRab4a\_S22N\_sens GCAGGAAGTGGCAAGAAACTGCTTACTTCATCAG  
HsRab4b\_S22N\_sens GCAGGAAGTGGCAAGAAACTGTCTCCTTCATCAG  
HsRab5a\_S34N\_sens TCCGCTGTTGGCAAAAACAGCCTAGTGCTTCGT  
HsRab5b\_S34N\_sens TCTGCAGTGGGAAAGAACAGCCTGGTATTACGT  
HsRab5c\_S35N\_sens TCTGCGGTAGGCAAAAACAGCCTCGTCCTCCGC  
HsRab6a\_T27N\_sens CAAAGCGTTGGAAAGAACTCTTTGATCACCAGA  
HsRab6b\_T27N\_sens CAGAGCGTCCGGAAGAAGAACTCTCTGATTACGAGG  
HsRab6c\_T27N\_sens CAAAGCGTTGCAAGAAGAACTCTTTGATCACCAGA  
HsRab7a\_T22N\_sens TCTGGAGTCCGGAAGAAGAACTCACTCATGAACAG  
HsRab7b\_T22N\_sens ATTGGTGTGGGAAAGAACTCCCTCCTTCACCAA  
HsRab8a\_T22N\_sens TCGGGGGTGGGGAAGAAGAACTGTGTCCTGTTCCGC  
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HsRab9a\_S21N\_sens GGTGGAGTTGGGAAGAAGAACTCACTTATGAACAGA  
HsRab9b\_S21N\_sens GGTGGAGTTGGGAAAAACTCGCTTATGAACCGT  
HsRab10\_T23N\_sens TCCGGAGTGGGGAAGAAGAACTGCGTCCTTTTTTCGT  
HsRab11a\_S25N\_sens TCTGGTGTGGAAAGAACAATCTCCTGTCTCGA  
HsRab11b\_S25N\_sens TCAGGCGTGGGCAAGAACAACCTGCTGTGCGCGC  
HsRab12\_T22N\_sens CTGGGCGCGGGCTCCAACGCGCTGTGCGGGCGGC  
HsRab13\_T22N\_sens TCGGGGGTGGGCAAGAAGAACTGTCTGATCATTCCG  
HsRab14\_S25N\_sens ATGGGAGTAGGAAAAAACTGCTTGCTTCATCAA  
HsRab15\_T22N\_sens TCCGGGGTGGGCAAGAAGAACTGCCTGCTGTGCCGC  
HsRab17\_S33N\_sens GGCTCCGTGGGTAAGAAGCAGCTTGGCTCTTCGG  
HsRab18\_S22N\_sens AGTGGGGTGGGCAAGAAGCAGCCTGCTCTTGAGG  
HsRab19\_T31N\_sens TCCAATGTGGGGAAGAAGAACTGTGTGGTGCAGCAT  
HsRab20\_T19N\_sens ATGAACGTGGGGAAGAAGAACTCGCTGCTGCAGCGG  
HsRab21\_T33N\_sens GGCTGCGTGGGGAAGAAGAACTCGCTGGTGTGCTGCGC  
HsRab22a\_S19N\_sens ACAGGTGTAGGTAAAAACAGTATTGTGTGGCGG  
HsRab23\_S23N\_sens GGAGCAGTTGGAAAAACAGTATGATTCAGCGA  
HsRab24\_T21N\_sens GAGTACGTGGGCAAGAAGCAGCCTGGTGGAGCGC  
HsRab25\_T26N\_sens TCAGGTGTGGGGAAGAACAATCTACTCTCCCGA  
HsRab26\_T77N\_sens TCGGGTGTGGGGAAGAAGAACTGTCTGCTGGTGGCA  
HsRab27a\_T23N\_sens TCTGGTGTAGGGAAGAAGCAGTGTACTTTACCAA  
HsRab27b\_T23N\_sens TCAGGGGTGGGGAAGAAGACATTTCTTTATAGA  
HsRab28\_T26N\_sens GGCGCCTCCGGGAAGAAGAACTCCTTAACTACGTGT  
HsRab29/Rab7L1\_T21N\_sens GCCGCAGTGGGCAAGAAGAACTCGCTGGTGCAGCGA  
HsRab30\_T23N\_sens GCTGGTGTGGGGAAGAAGAACTGCCTCGTCCGAAGA  
HsRab31\_S20N\_sens ACTGGGGTGGGAAAAACAGCATCGTGTGTCTCGA  
HsRab32\_T39N\_sens CTTGGCGTGGGCAAGAAGCAGCATCATCAAGCGC

HsRab33a_T50N_sens	TCCAACGTGGGCAAGAAGTGCCTGACCTTCCGC
HsRab33b_T47N_sens	TCCAATGTGGGCAAGAAGTGCCTGACCTACCGC
HsRab34_T66N_sens	CTGTCGGTGGGGAAGAAGTGCCTCATTAATAGG
HsRab35_S22N_sens	AGCGGTGTGGGCAAGAACAGTTTACTGTTGCGT
HsRab36_T137N_sens	CTCTACGTGGGGAAGAACAGCCTCATCCACAGG
HsRab37_T43N_sens	ACAGGCGTGGGCAAAAAGTGTTCCTGATCCAA
HsRab38_T23N_sens	CTGGGCGTGGGGAAGAACAGTATCATCAAGCGC
HsRab39a_S22N_sens	TCCACCGTGGGCAAGAAGTGCCTCCTGCACCGC
HsRab39b_S22N_sens	TCCACAGTGGGCAAGAAGTGCCTGATCCGCCGC
HsRab40a_S28N_sens	AGGGACGTAGGCAAGAACGAGATCCTGGAGAGC
HsRab40b_G28N_sens	AGCGACGTGGGCAAGAACGAGATCCTGGCGAGC
HsRab40c_G28N_sens	AGCGACGTGGGCAAGAACGAGATCCTGGAGAGC
HsRab41_T44N_sens	GAGCAGAGCGGGAAGAAGTCCATCATCAGCCGC
rab42_N_sens	GCAAGCGTGGGCAAGAAGTGCCTGGTGCAGCGC

**Supplemental Table 3**

<b>siRNA</b>	<b>Sequence</b>
siLuc	5' - CGUACGCGGAAUACUUCGA -3'
siMyoVa3	5' - CGAAACAACUGGAACUCGA - 3'
siMyoVa4	5' - CAAUAUGAGAACUUAUCUU - 3'
siRab3D	5' - GUUCAAACUGCUACUGAUA -3'
siRab6A/A'	5' - GACAUCUUUGAUCACCAGA -3'
siRab8A	5' - CAGCUUUUCCGAUGUGUU -3'
siRab10	5' - GAAUAGACUUCAAGAUCAA -3'
siRab11A/B	5' - GACGACGAGUACGACUACC -3'
siRab14	5' - CAACUACUCUUACAUCUUU - 3'