Supplemental Materials

Molecular Biology of the Cell

Zhao et al.

Supplemental Figure S1. Strategy for screening recessive mutations on 2L.

(A) The second chromosome of *ey-flp,Rh1-GFP;FTR40A* flies was isogenized and flies were mutagenized by feeding 25 mM EMS (Sigma) in 2% sucrose for 8 h. They were then mated to *ey-flp Rh1-GFP;GMR-hid CL FRT40A/Cyo hs-hid* flies. Flies were heat shocked at 37°C for 1 h to avoid having heterozygous flies among the F1 progeny (the pro-apoptosis gene *hid* was induced by heat-shock (*hs-hid*) in heterozygous flies). The DPP was assessed in F1 flies using a fluorescent stereo microscope day 1 and 5 following eclosion.

Supplemental Figure S2. Alignment of different *Drosophila* Vha68 proteins.

Amino acid sequences of *Drosophila* Vha68-1, Vha68-2, and Vha68-3, as well as human ATP6V1A are shown. Identical residues, found in at least two proteins, are enclosed in black boxes. The running tally of amino acids is indicated to the right.

Supplemental Figure S3. Decreased light sensitivity and reduced Rh1 levels in $vha68\text{-}1^{1}$ flies.

(A) Light sensitivity assay was performed using ~1-day-old wild-type, $vha68\text{-}1^{1}$, and $vha100\text{-}1^{2}$ flies. Flies were dark-adapted for 2 min before being exposed to five pulses of 5-sec of white light with increasing intensity. The maximal light intensity was 300 Lux (10^{-1}). (B) Total Rh1 levels were decreased in $vha68\text{-}1^{1}$ flies. Head extracts were prepared from 1-day-old flies of indicated genotypes and labeled using antibodies against Rh1 and INAD. (C) Quantification of Rh1 levels in each genotype. Rh1 levels were normalized to INAD levels, which served as a loading control. *n* = 7, data are presented as mean ± SD, ***p < 0.001 (Student's unpaired t-test).

Supplemental Figure S4. Localization of the scaffold protein INAD and the basolateral membrane protein $Na^{+}K^{+}$ATPase were not affected in $vha68\text{-}1^{1}$ flies.

(A) Tangential resin-embedded retina sections from ~1-day-old wild type and *vha68-1*^{1} flies were labeled using antibodies against Rh1 (green) and INAD (red). The localization pattern of INAD was indistinguishable between control and *vha68-1*^{1} flies. Scale bars are 2 μm. (B) Adult eyes from wild type and *vha68-1*^{1} flies were dissected and stained with antibodies against $Na^{+}K^{+}$ATPase (red). GFP fluorescence of Rh1-GFP was directly observed to indicate rhabdomeres. The localization pattern of $Na^{+}K^{+}$ATPase was not affected in *vha68-1*^{1} flies. Scale bars are 2 μm.

Supplemental Figure S5. Schematic view and representative images of the Rh1 pulse-chase assay.

(A) Schematic shows the Rh1 pulse-chase assay. Newly hatched flies were exposed to 37°C for 1 h, and then placed in the dark. Flies were collected at indicated time points (6, 18, 24, and 48 h) after heat-shock. Rh1-GFP localized in the rhabdomere, marked by green; hs-Rh1-RFP was synthesized in the ER and transported to the rhabdomere, marked by red. N, nuclear; C, Cell body; R, rhabdomere. (B-E) Cryostat sections of *hs-Rh1-RFP/+* and *vha68-1*^{1}*;hs-Rh1-RFP/+* heads at 6 (B), 18 (C), 24 (D), and 48 h (E) after heat shock were labeled with antibodies against RFP. GFP fluorescence of Rh1-GFP was directly observed to indicate rhabdomeres. Scale bars are 5 μm.

Supplemental Figure S6. Vha68-1 functions in Rh1 post-Golgi trafficking to rhabdomere.

(A) Cryostat sections of *ninaE-gal4;UAS-shi*^{ts1} (*ey-flp rh1-GFP;ninaE-gal4/+;hs-rh1-RFP/UAS-shi*^{ts1}) , *vha68-1*^{1} (*ey-flp rh1-GFP;vha68-1*^{1} *FRT40A/GMR-hid CL FRT40A; hs-rh1-RFP /+*) and *vha68-1*^{1}*ninaE-gal4;UAS-shi*^{ts1} (*ey-flp rh1-GFP; vha68-1*^{1} *FRT40A ninaE-gal4/GMR-hid CL FRT40A;hs-rh1-RFP/UAS-shi*^{ts1}) heads 24 h after heat shock were labeled with antibodies against RFP. GFP fluorescence of Rh1-GFP was directly observed to indicate rhabdomeres. Scale bars are 5 μm. (B) Quantification of rhabdomere-localized, newly synthesized Rh1-RFP at 24 h after heat shock. Six different compound eye sections of each sample were quantified. Error bars indicate SD, ***p < 0.001; ns, not significant (Student's unpaired t-test). (C) Adult eyes from wild type, *vha68-1*^{1}, *ninaE-gal4;UAS-shi*^{ts1}, and *vha68-1*^{1}*ninaE-gal4;UAS-shi*^{ts1} flies were dissected and stained with antibodies against Rh1 (green). Rh1 vesicles still accumulated in *vha68-1*^{1} flies in the *shi*^{ts1} background. Scale bars are 5 μm.

Supplemental Figure S7. Vha100-1 colocalizes with multiple endosome markers in the synaptic terminal in the lamina.

(A) The nSyb-mp reporter and Vha100-1 colocalize in the synaptic terminal in the lamina. Cryostat sections of *nSyb-mp/v100-myc* (*ninaE-nSyb-mCherry-pHluorin/ninaE-vha100-myc*) were labeled with antibodies against Myc (green) and RFP (red). Scale bars are 5 μm. (B) Cryostat sections of *rab5-RFP/v100-myc* (*ninaE-rab5-RFP/ninaE-vha100-myc*), *rab7-RFP/v100-myc* (*ninaE-rab7-RFP/ninaE-vha100-myc*), *rab11-RFP/v100-myc* (*ninaE-rab11-RFP/ninaE-vha100-myc*), and *lamp1-RFP/v100-GFP* (*ninaE-lamp1-RFP/ninaE-vha100-GFP*) heads were labeled with antibodies against Myc (green) or GFP (green) and RFP (red). Scale bars are 5 μm.

Supplemental Figure S8. V-ATPase is required to maintain pH in Rh1 secretory vesicles during its trafficking to the rhabdomere and the deglycosylation process.

(A) Tangential resin-embedded retina sections of compound eyes from ~1-day-old wild type, *dmppe*^{e02905}, *vha68-1*^{1}, and *vha68-1*^{1} *dmppe*^{e02905} flies were labeled using antibodies against Rh1. Scale bars are 2 μm. (B) Tangential resin-embedded retina sections of compound eyes from ~1-day-old wild type, *vha100-1*^{2}, *ninaE-v100-GFP;vha100-1*^{2} (*ey-flp rh1-GFP; ninaE-vha100-1-GFP/+;vha100-1*^{2} *FRT82B/GMR-hid CL FRT82B*), and *ninaE-v100*^{R755A}*-GFP;vha100-1*^{2} (*ey-flp rh1-GFP; ninaE-vha100-1*^{R755A}*-GFP/+;vha100-1*^{2} *FRT82B/GMR-hid CL FRT82B*) flies were labeled using antibodies against Rh1. Scale bars are 2 μm. (C) Head extracts were prepared from ~1-day-old wild type, *vha100-1*^{2}, *ninaE-v100-GFP;vha100-1*^{2}, and *ninaE-v100*^{R755A}*-GFP;vha100-1*^{2} flies and labeled with antibodies against Rh1.

V-ATPase subunit	**mutation**	**phenotype**
vha100	*vha100-1*^{1}	No ERG transient
vha100	*vha100-1*^{2}	No ERG transient
vhaSFD	*vhaSFD*^{EY04644}	No homozygous eye
vha68-2	*vha68-2*^{S4214}	No homozygous eye
vha68-2	*vha68-2*^{EP2364}	No homozygous eye
vha68-2	*vha68-2*^{R6}	No homozygous eye
vha44	*vha44*^{KG00915}	No homozygous eye
vhaAC39-1	*vhaAC39-1*^{FY38}	No homozygous eye
vhaAC39-1	*vhaAC39-1*^{FZ29}	No homozygous eye

Table S1: Phenotypes of mutations in V-ATPase genes. All alleles are homozygous lethal, and eyes ofhomozygous mutationswere generateusing the“*Rh1::GFPey-flp/hid*” method.

Figure S1

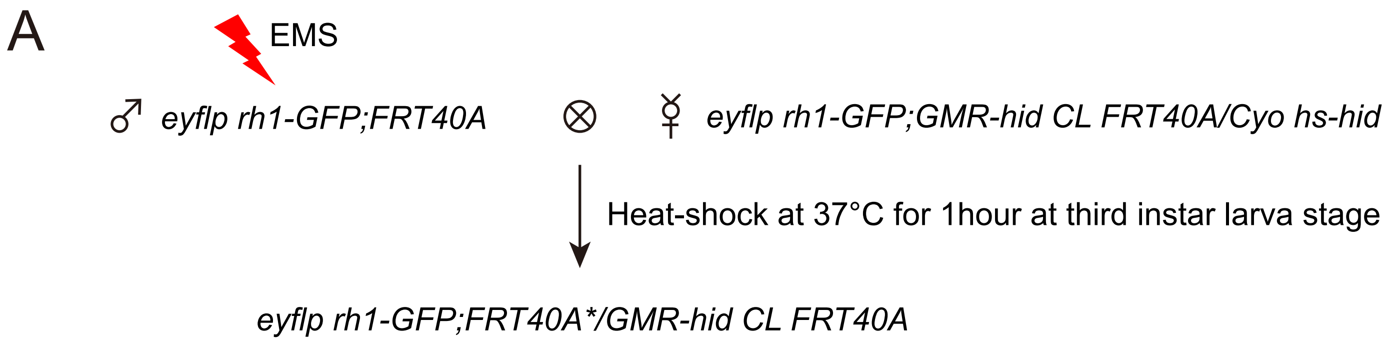

A
EMS
♂ eyflp rh1-GFP;FRT40A
⊗
☿ eyflp rh1-GFP;GMR-hid CL FRT40A/Cyo hs-hid
Heat-shock at 37°C for 1hour at third instar larva stage
eyflp rh1-GFP;FRT40A*/GMR-hid CL FRT40A

Figure S2

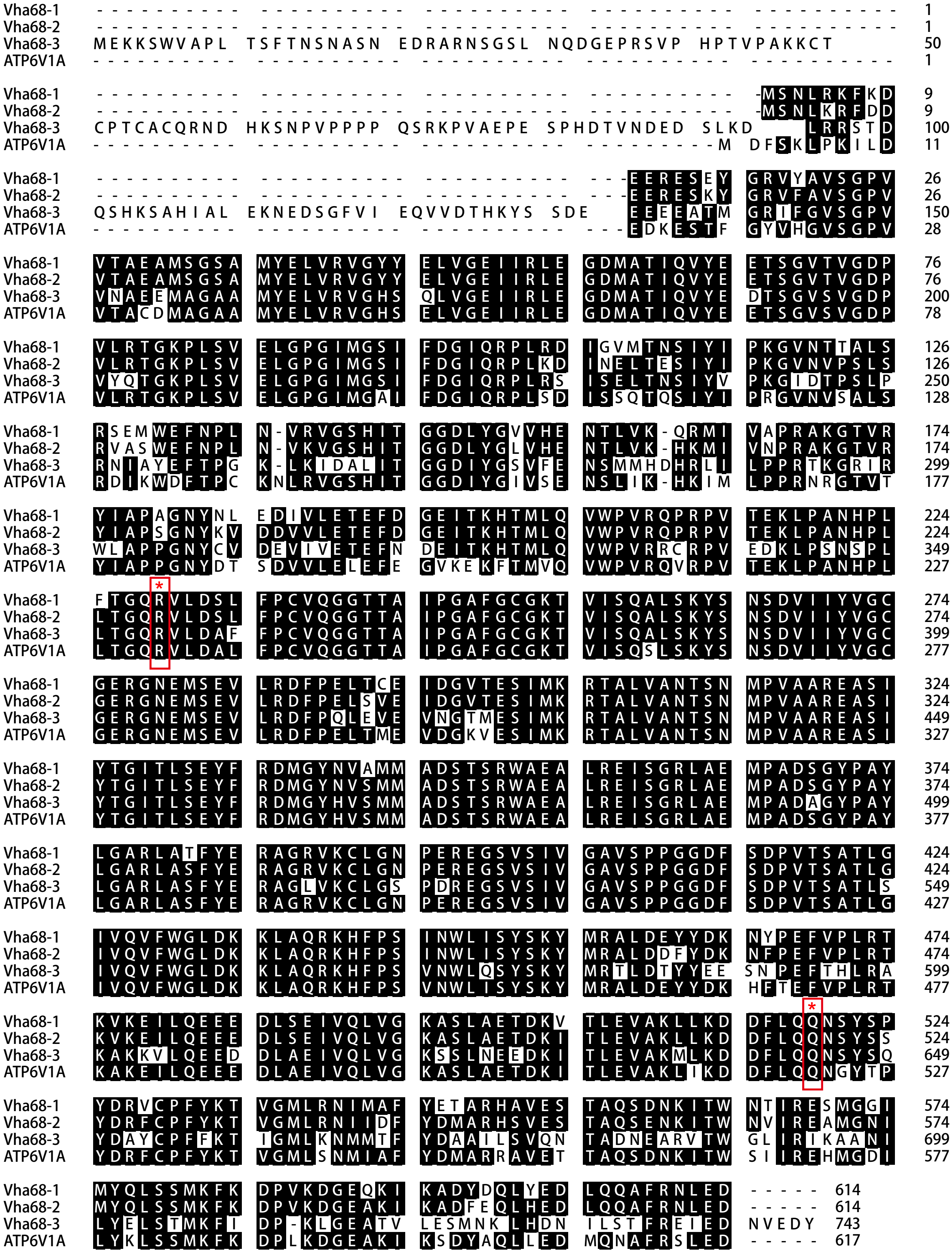

Vha68-1 ---------- ---------- ---------- ---------- ---------- 1
Vha68-2 ---------- ---------- ---------- ---------- ---------- 1
Vha68-3 MEKKSWVAPL TSFTNSNASN EDRARNSGSL NQDGEPRSVP HPTVPAKKCT 50
ATP6V1A ---------- ---------- ---------- ---------- ---------- 1
Vha68-1 ---------- ---------- ---------- ---------- -MSNLRKFKD 9
Vha68-2 ---------- ---------- ---------- ---------- -MSNLKRFDD 9
Vha68-3 CPTCACQRND HKSNPVPPPP QSRKPVAEPE SPHDTVNDED SLKD LRRSTD 100
ATP6V1A ---------- ---------- ---------- ---------M DFSKLPKILD 11
Vha68-1 ---------- ---------- ---------- ---EERESEY GRVYAVSGPV 26
Vha68-2 ---------- ---------- ---------- ---EERESKY GRVFAVSGPV 26
Vha68-3 QSHKSAHIAL EKNEDSGFVI EQVVDTHKYS SDE EEEEATM GRIFGVSGPV 150
ATP6V1A ---------- ---------- ---------- ---EDKESTF GYVHGVSGPV 28
Vha68-1 VTAEAMSGSA MYELVRVGYY ELVGEIIRLE GDMATIQVYE ETSGVTVGDP 76
Vha68-2 VTAEAMSGSA MYELVRVGYY ELVGEIIRLE GDMATIQVYE ETSGVTVGDP 76
Vha68-3 VNAEEMAGAA MYELVRVGHS QLVGEIIRLE GDMATIQVYE DTSGVSVGDP 200
ATP6V1A VTACDMAGAA MYELVRVGHS ELVGEIIRLE GDMATIQVYE ETSGVSVGDP 78
Vha68-1 VLRTGKPLSV ELGPGIMGSI FDGIQRPLRD IGVMTNSIYI PKGVNTTALS 126
Vha68-2 VLRTGKPLSV ELGPGIMGSI FDGIQRPLKD INELTESIYI PKGVNVPSLS 126
Vha68-3 VYQTGKPLSV ELGPGIMGSI FDGIQRPLRS ISELTNSIYV PKGIDTPSLP 250
ATP6V1A VLRTGKPLSV ELGPGIMGAI FDGIQRPLSD ISSQTQSIYI PRGVNVSALS 128
Vha68-1 RSEMWEFNPL N-VRVGSHIT GGDLYGVVHE NTLVK-QRMI VAPRAKGTVR 174
Vha68-2 RVASWEFNPL N-VKVGSHIT GGDLYGLVHE NTLVK-HKMI VNPRAKGTVR 174
Vha68-3 RNIAYEFTPG K-LKIDALIT GGDIYGSVFE NSMMHDHRLI LPPRTKGRIR 299
ATP6V1A RDIKWDFTPC KNLRVGSHIT GGDIYGIVSE NSLIK-HKIM LPPRNRGTVT 177
Vha68-1 YIAPAGNYNL EDIVLETEFD GEITKHTMLQ VWPVRQPRPV TEKLPANHPL 224
Vha68-2 YIAPSGNYKV DDVVLETEFD GEITKHTMLQ VWPVRQPRPV TEKLPANHPL 224
Vha68-3 WLAPPGNYCV DEVIVETEFN DEITKHTMLQ VWPVRRCRPV EDKLPSNSPL 349
ATP6V1A YIAPPGNYDT SDVVLELEFE GVKEKFTMVQ VWPVRQVRPV TEKLPANHPL 227
*
Vha68-1 FTGQRVLDSL FPCVQGGTTA IPGAFGCGKT VISQALSKYS NSDVIIYVGC 274
Vha68-2 LTGQRVLDSL FPCVQGGTTA IPGAFGCGKT VISQALSKYS NSDVIIYVGC 274
Vha68-3 LTGQRVLDAF FPCVQGGTTA IPGAFGCGKT VISQALSKYS NSDVIIYVGC 399
ATP6V1A LTGQRVLDAL FPCVQGGTTA IPGAFGCGKT VISQSLSKYS NSDVIIYVGC 277
Vha68-1 GERGNEMSEV LRDFPELTCE IDGVTESIMK RTALVANTSN MPVAAREASI 324
Vha68-2 GERGNEMSEV LRDFPELSVE IDGVTESIMK RTALVANTSN MPVAAREASI 324
Vha68-3 GERGNEMSEV LRDFPQLEVE VNGTMESIMK RTALVANTSN MPVAAREASI 449
ATP6V1A GERGNEMSEV LRDFPELTME VDGKVESIMK RTALVANTSN MPVAAREASI 327
Vha68-1 YTGITLSEYF RDMGYNVAMM ADSTSRWAEA LREISGRLAE MPADSGYPAY 374
Vha68-2 YTGITLSEYF RDMGYNVSMM ADSTSRWAEA LREISGRLAE MPADSGYPAY 374
Vha68-3 YTGITLSEYF RDMGYHVSMM ADSTSRWAEA LREISGRLAE MPADAGYPAY 499
ATP6V1A YTGITLSEYF RDMGYHVSMM ADSTSRWAEA LREISGRLAE MPADSGYPAY 377
Vha68-1 LGARLATFYE RAGRVKCLGN PEREGSVSIV GAVSPPGGDF SDPVTSATLG 424
Vha68-2 LGARLASFYE RAGRVKCLGN PEREGSVSIV GAVSPPGGDF SDPVTSATLG 424
Vha68-3 LGARLASFYE RAGLVKCLGS PDREGSVSIV GAVSPPGGDF SDPVTSATLS 549
ATP6V1A LGARLASFYE RAGRVKCLGN PEREGSVSIV GAVSPPGGDF SDPVTSATLG 427
Vha68-1 IVQVFWGLDK KLAQRKHFPS INWLISYSKY MRALDEYYDK NYPEFVPLRT 474
Vha68-2 IVQVFWGLDK KLAQRKHFPS INWLISYSKY MRALDDFYDK NFPEFVPLRT 474
Vha68-3 IVQVFWGLDK KLAQRKHFPS VNWLQSYSKY MRTLDTYYEE SNPEFTHLRA 599
ATP6V1A IVQVFWGLDK KLAQRKHFPS VNWLISYSKY MRALDEYYDK HFTEFVPLRT 477
*
Vha68-1 KVKEILQEEE DLSEIVQLVG KASLAETDKV TLEVAKLLKD DFLQQNSYSP 524
Vha68-2 KVKEILQEEE DLSEIVQLVG KASLAETDKI TLEVAKLLKD DFLQQNSYSS 524
Vha68-3 KAKKVLQEED DLAEIVQLVG KSSLNEEDKI TLEVAKMLKD DFLQQNSYSQ 649
ATP6V1A KAKEILQEEE DLAEIVQLVG KASLAETDKI TLEVAKLIKD DFLQQNGYTP 527
Vha68-1 YDRVCPFYKT VGMLRNIMAF YETARHAVES TAQSDNKITW NTIRESMGGI 574
Vha68-2 YDRFCPFYKT VGMLRNIIDF YDMARHSVES TAQSENKITW NVIREAMGNI 574
Vha68-3 YDAYCPFFKT IGMLKNMMTF YDAAILSVQN TADNEARVTW GLIRIKAANI 699
ATP6V1A YDRFCPFYKT VGMLSNMIAF YDMARRAVET TAQSDNKITW SIIREHMGDI 577
Vha68-1 MYQLSSMKFK DPVKDGEQKI KADYDQLYED LQQAFRNLED ----- 614
Vha68-2 MYQLSSMKFK DPVKDGEAKI KADFEQLHED LQQAFRNLED ----- 614
Vha68-3 LYELSTMKFI DP-KLGEATV LESMNKLHDN ILSTFREIED NVEDY 743
ATP6V1A LYKLSSMKFK DPLKDGEAKI KSDYAQLLED MQNAFRSLED ----- 617

Figure S3

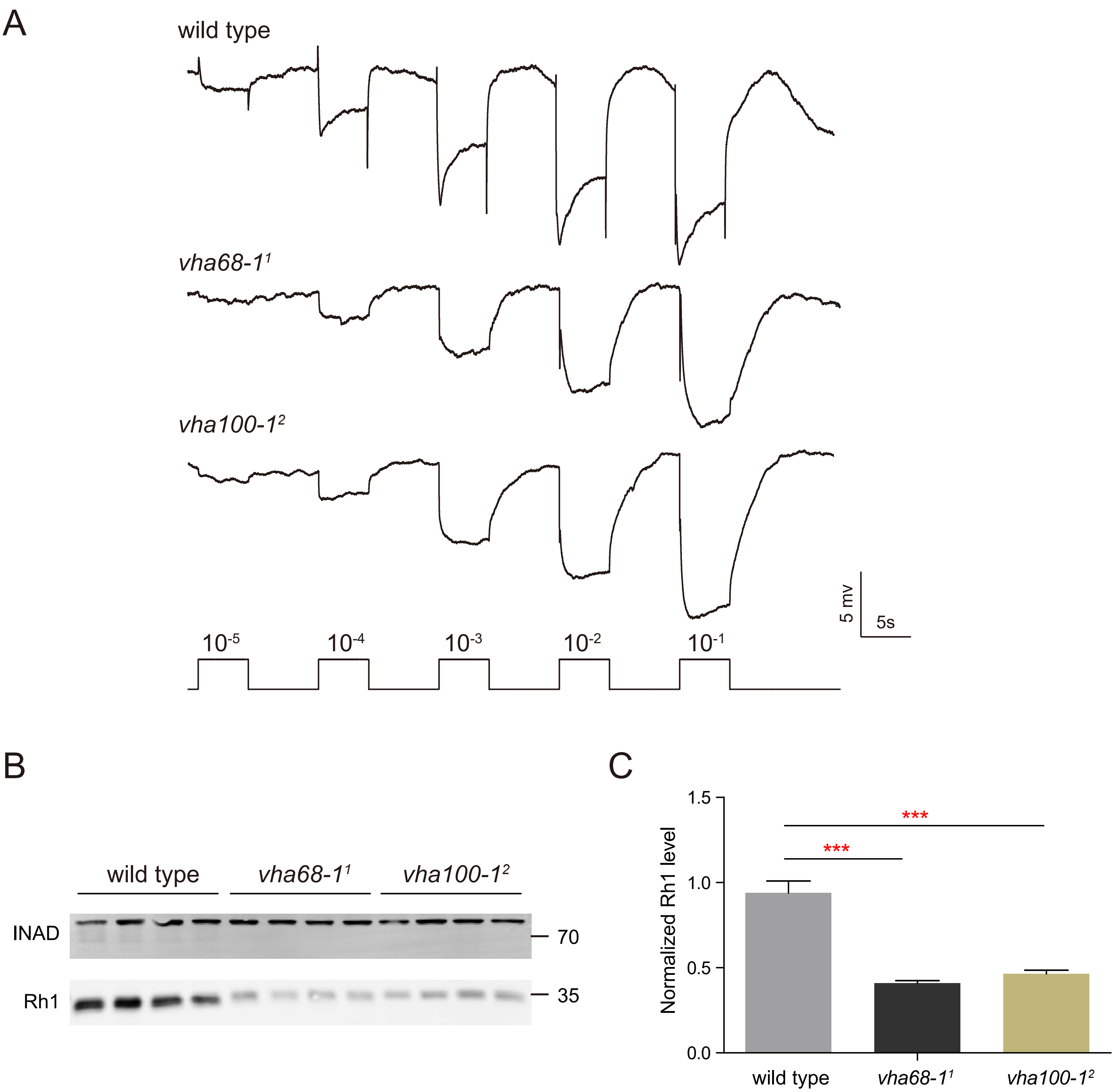

A
wild type
vha68-1^{1}
vha100-1^{2}
5 mv
5s
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
B
wild type
vha68-1^{1}
vha100-1^{2}
INAD
Rh1
70
35
C
Normalized Rh1 level
1.5
1.0
0.5
0.0
***
***
wild type
vha68-1^{1}
vha100-1^{2}

Figure S4

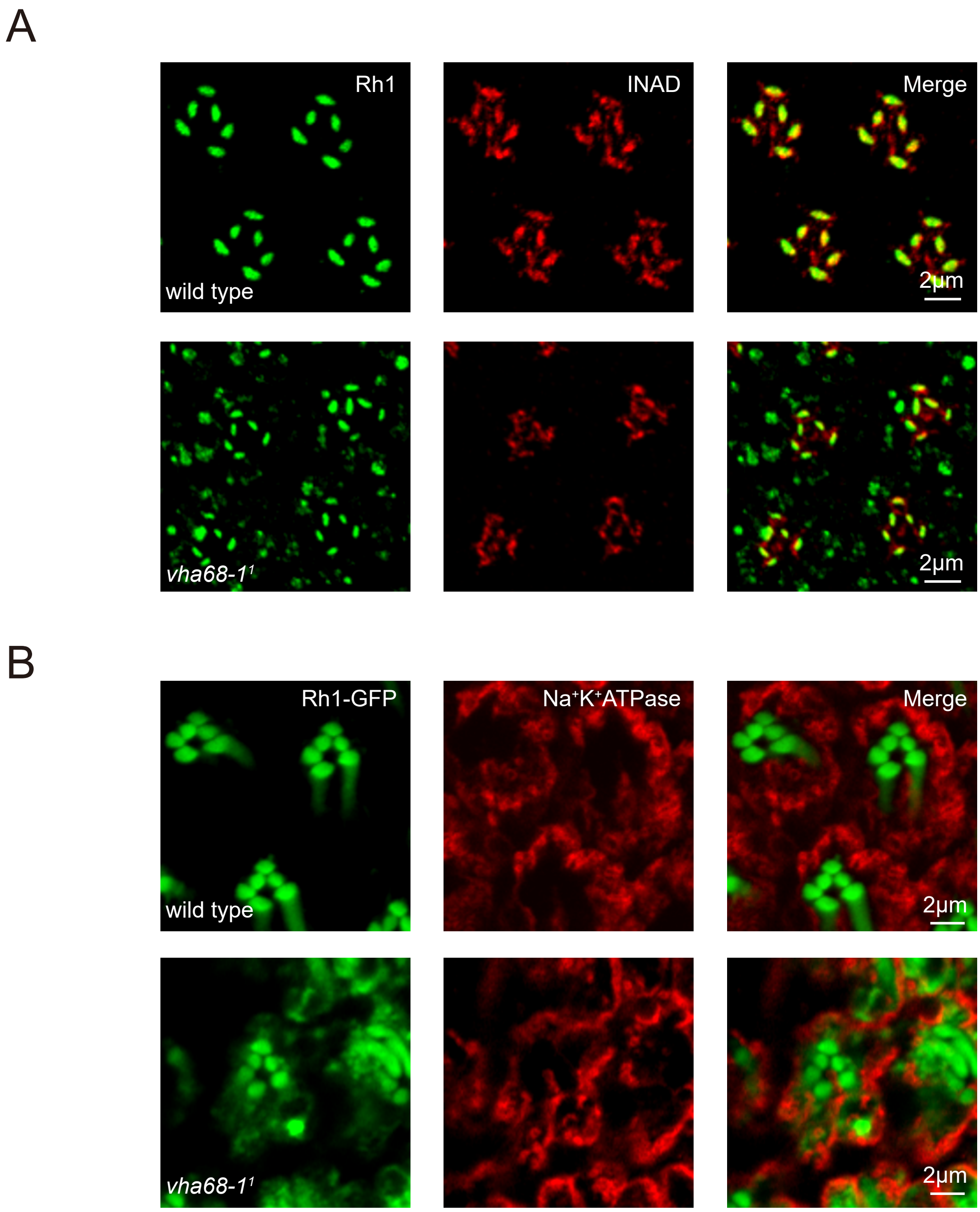

A
Rh1
INAD
Merge
wild type
2μm
vha68-1[1]
2μm
B
Rh1-GFP
Na⁺K⁺ATPase
Merge
wild type
2μm
vha68-1[1]
2μm

Figure S5

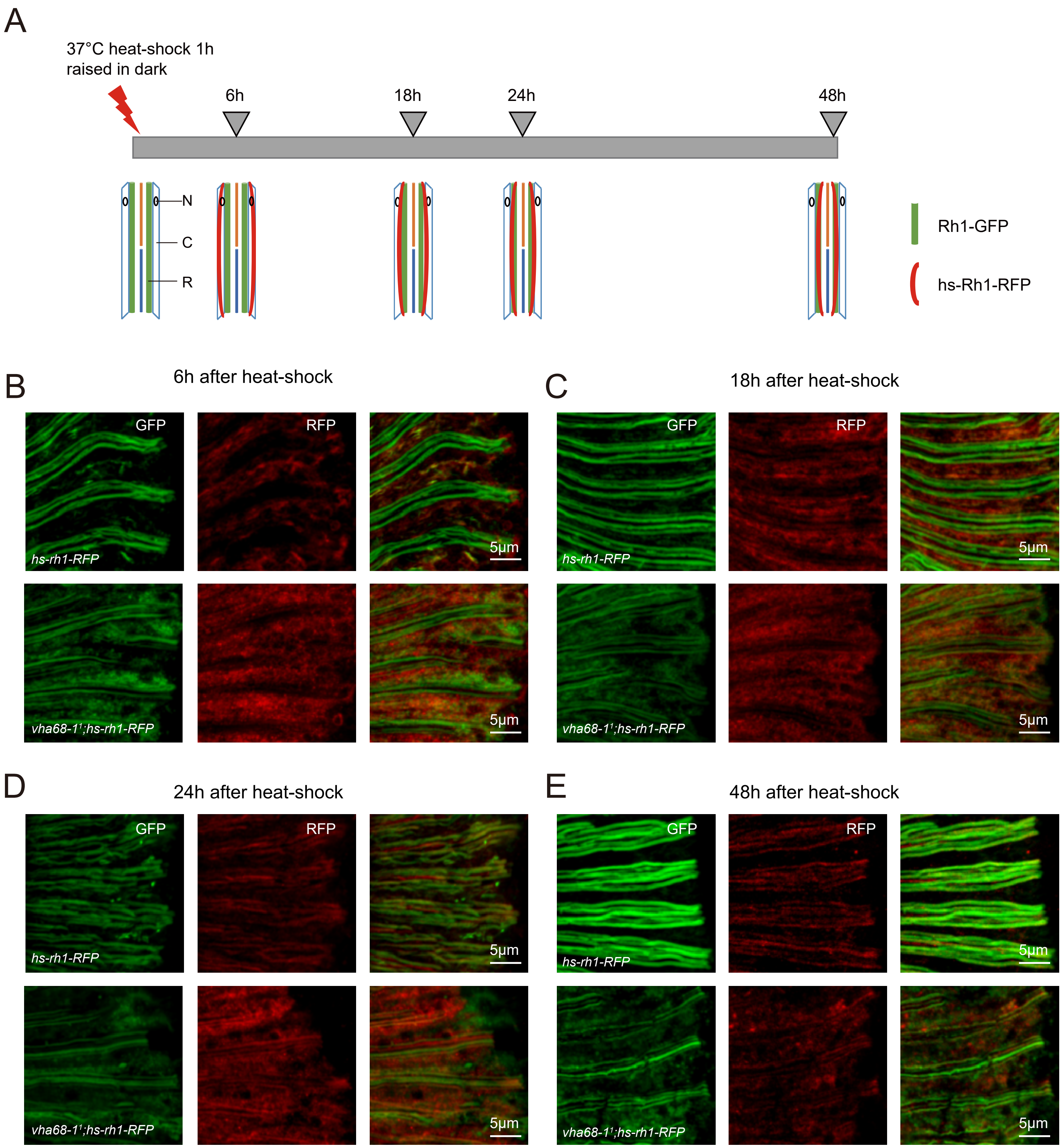
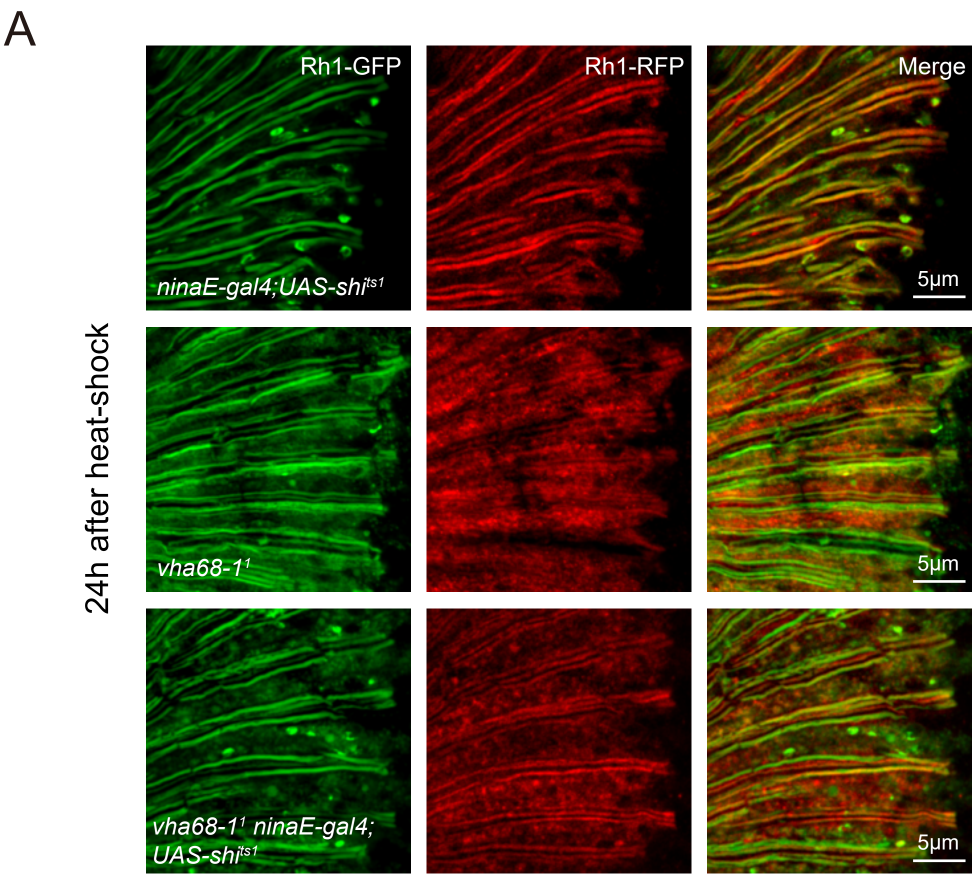
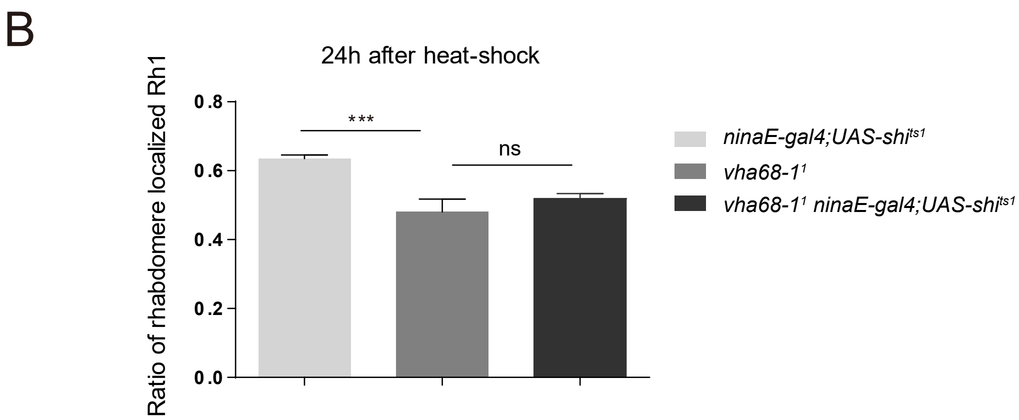

A
37°C heat-shock 1h
raised in dark
6h
18h
24h
48h
N
C
R
Rh1-GFP
hs-Rh1-RFP
B
6h after heat-shock
GFP
RFP
hs-rh1-RFP
vha68-1[1];hs-rh1-RFP
5μm
C
18h after heat-shock
D
24h after heat-shock
E
48h after heat-shock

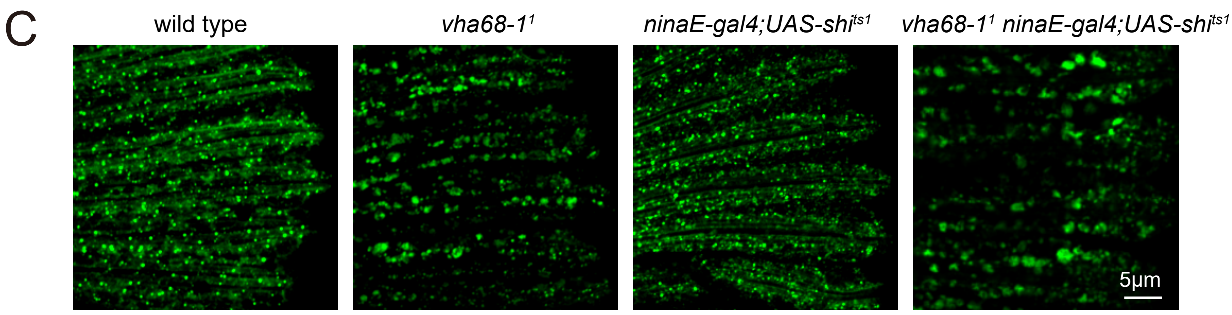
Figure S6



A
24h after heat-shock
Rh1-GFP
Rh1-RFP
Merge
ninaE-gal4;UAS-shi^ts1
vha68-1^1
vha68-1^1 ninaE-gal4;
UAS-shi^ts1
5μm
5μm
5μm



B
24h after heat-shock
Ratio of rhabdomere localized Rh1
0.8
0.6
0.4
0.2
0.0
***
ns
ninaE-gal4;UAS-shi^ts1
vha68-1^1
vha68-1^1 ninaE-gal4;UAS-shi^ts1



C
wild type
vha68-1^1
ninaE-gal4;UAS-shi^ts1
vha68-1^1 ninaE-gal4;UAS-shi^ts1
5μm

Figure S7

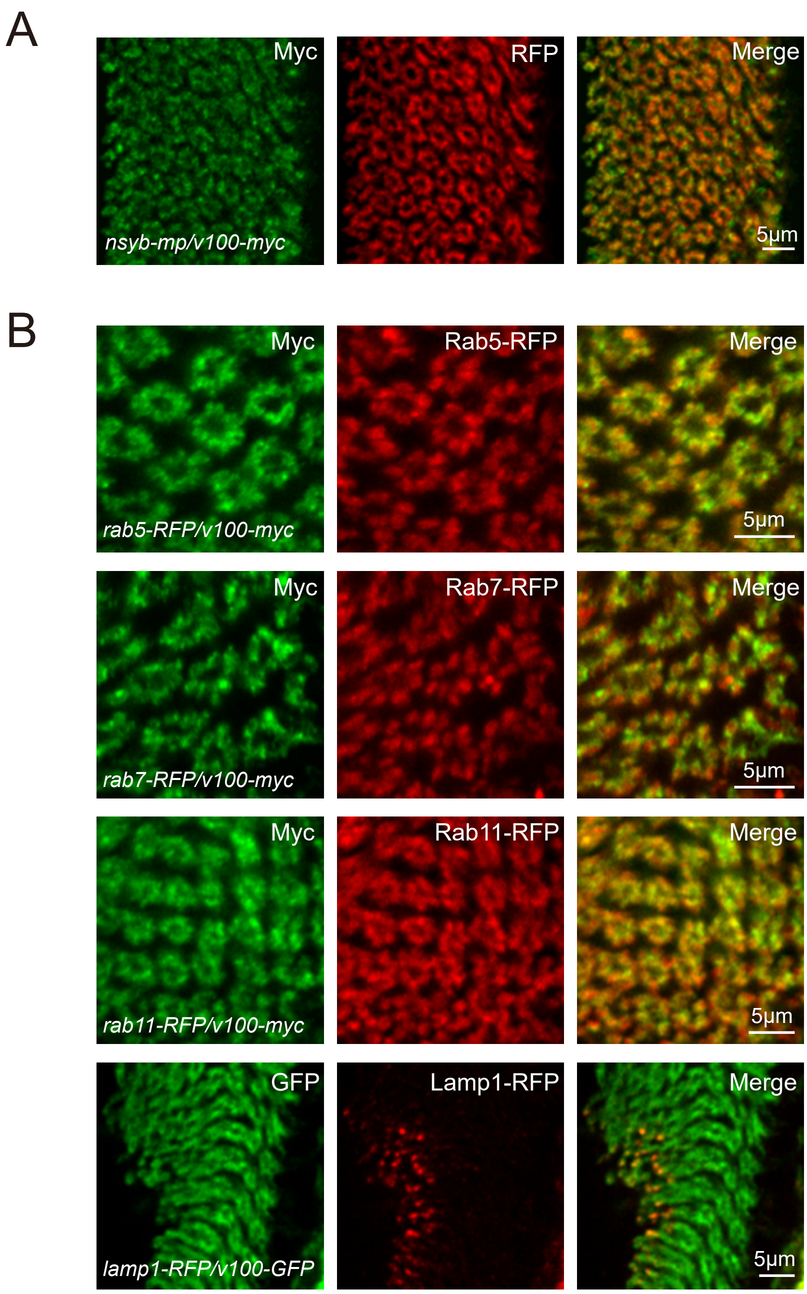
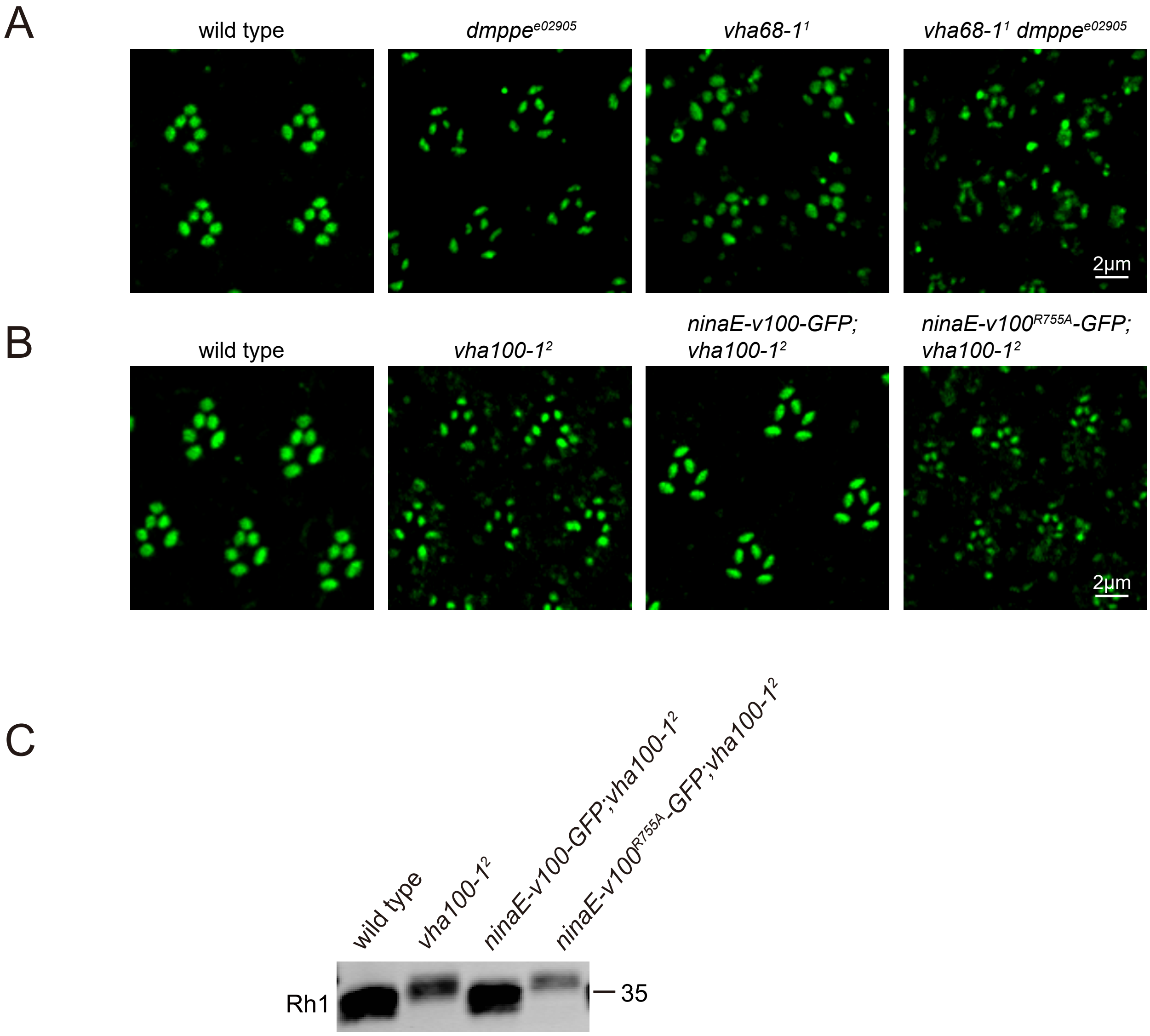

A
Myc
RFP
Merge
nsyb-mp/v100-myc
5µm
B
Myc
Rab5-RFP
Merge
rab5-RFP/v100-myc
5µm
Myc
Rab7-RFP
Merge
rab7-RFP/v100-myc
5µm
Myc
Rab11-RFP
Merge
rab11-RFP/v100-myc
5µm
GFP
Lamp1-RFP
Merge
lamp1-RFP/v100-GFP
5µm

Figure S8



A
wild type
dmppe e02905
vha68-1 1
vha68-1 1 dmppe e02905
2μm
B
wild type
vha100-1 2
ninaE-v100-GFP; vha100-1 2
ninaE-v100 R755A-GFP; vha100-1 2
2μm
C
wild type
vha100-1 2
ninaE-v100-GFP;vha100-1 2
ninaE-v100 R755A-GFP;vha100-1 2
Rh1
35