Mrozowska and Fukuda, http://www.jcb.org/cgi/content/full/jcb.201512024/DC1

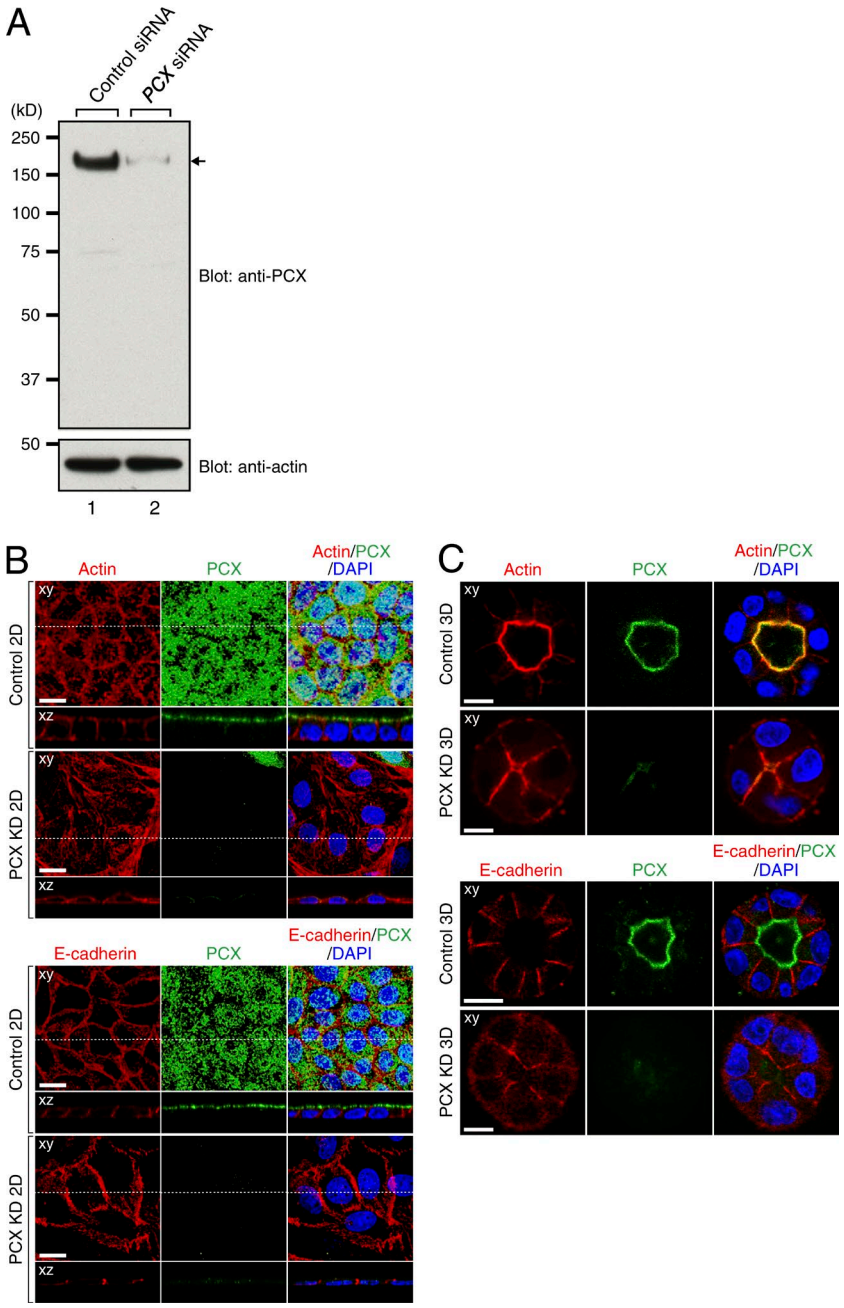

Figure S1. **Specificity of anti-PCX antibody and morphology of PCX-KD cells.** (A) MDCK II cells that had been treated with control siRNA or *PCX* siRNA were lysed, and their cell lysates were analyzed by immunoblotting with anti-PCX antibody and anti-actin antibody. Note that the anti-PCX antibody specifically recognized a single immunoreactive band with an apparent molecular mass of 170 kD (lane 1, arrow) and that the *PCX* siRNA treatment dramatically decreased the band intensity (lane 2). The positions of the molecular mass markers (in kilodaltons) are shown on the left. (B and C) MDCK II cells that had been treated with control siRNA or *PCX* siRNA were plated on glass-bottom dishes (B) or on Matrigel-coated glass slides (C) and fixed with PFA after 48 h (B) or 72 h (C). The cells were then stained with anti-PCX antibody (green), Texas red–conjugated phalloidin (or anti-E-cadherin antibody; red), and DAPI (blue). Note that even though PCX KD prevents lumen formation in 3D cysts, the cells are still polarized. In B, the confocal xy section (top) and the xz section (bottom), which corresponds to the dashed line in the xy section, of 2D monolayers are shown. Bars, 10 µm.

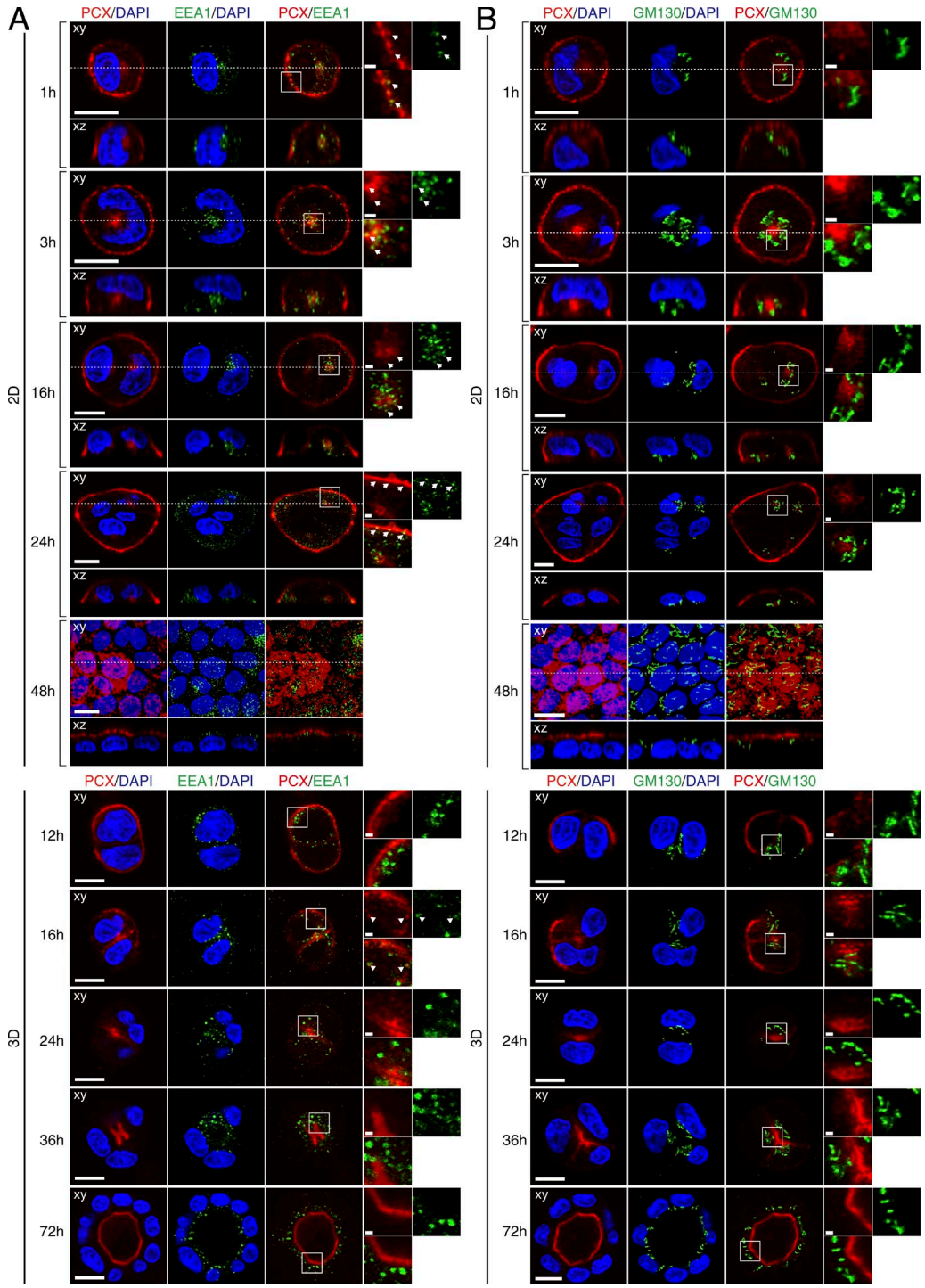

Figure S2. **PCX colocalization with organelle markers in 2D monolayers and in 3D cysts.** MDCK II cell cultures and immunostainings were performed as described in the legend of Fig. 2. Coimmunostaininig between PCX (red), DAPI (blue), and (A) EEA1, (B) GM130, or (C) TfR (green) is shown. The confocal xy section (top) and the xz section (bottom), which corresponds to the dashed line in the xy section, of 2D cell cultures are shown. The fourth columns show magnifications of the boxed regions in the third columns. The arrows in A show the colocalization points between EEA1 and PCX. Localization of PCX (red) in each time point is illustrated schematically on the right side of each panel in C. Bars: 10 µm; (insets) 1 µm. Panel C is not depicted because of space limitations, but it is available in the JCB DataViewer at http://dx.doi.org/10.1083/jcb.201512024.dv/.

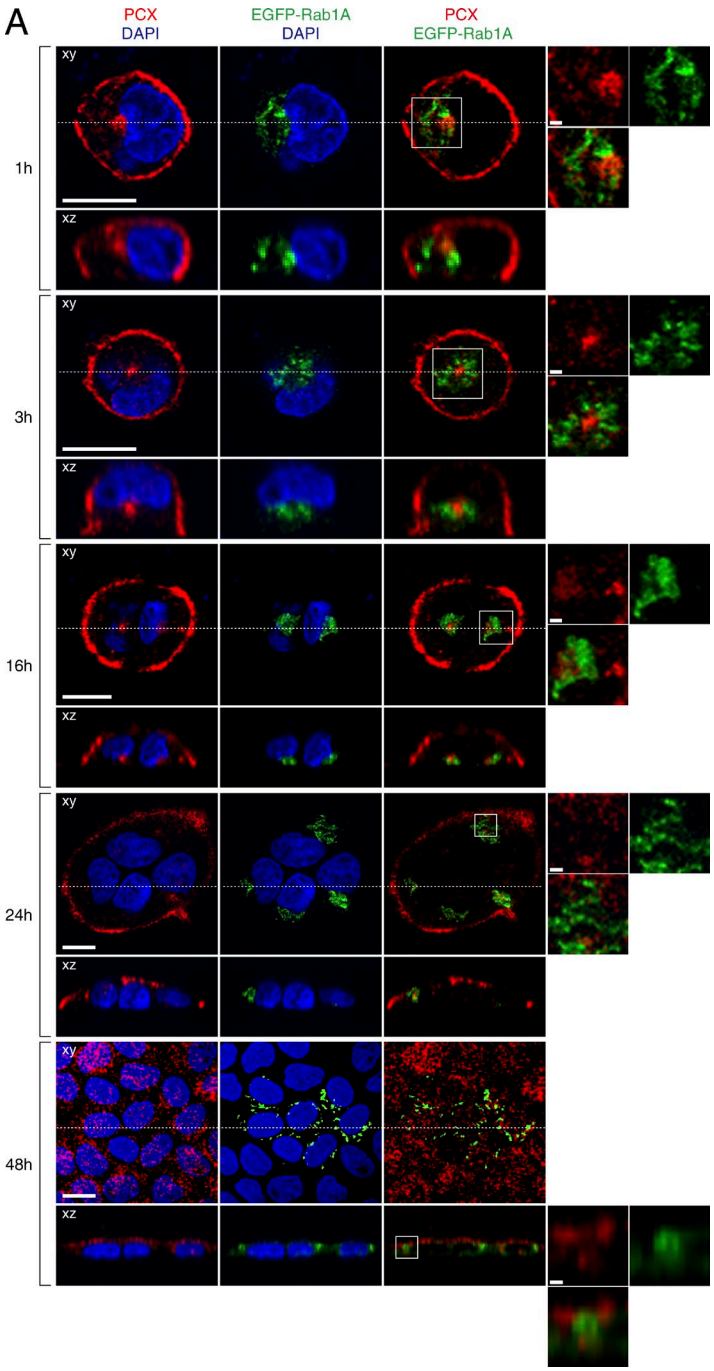

Figure S3. **Colocalization of endogenous PCX with EGFP-tagged Rab GTPases in 2D MDCK II cells.** MDCK II cells were transfected with a series of plasmids carrying EGFP-tagged Rab GTPases from Rab1A to Rab43 (AA–CE), with the exception of Rab40C (CB), which was Myc tagged. Cells were then plated on glass-bottom dishes and fixed with PFA at the times indicated (see also Fig. 3). After that cells were stained with anti-PCX antibody (red) and DAPI (blue). The confocal xy section (top) and the xz section (bottom), which corresponds to the dashed line in the xy section, are shown. The fourth columns show magnifications of the boxed regions in the third columns. The arrows in the right columns show the colocalization points between PCX and Rabs. Bars: 10 µm; (insets) 1 µm. Panels AB–CE are not depicted here because of space limitations, but they are fully available in the JCB DataViewer at http://dx.doi.org/10.1083/jcb.201512024.dv.

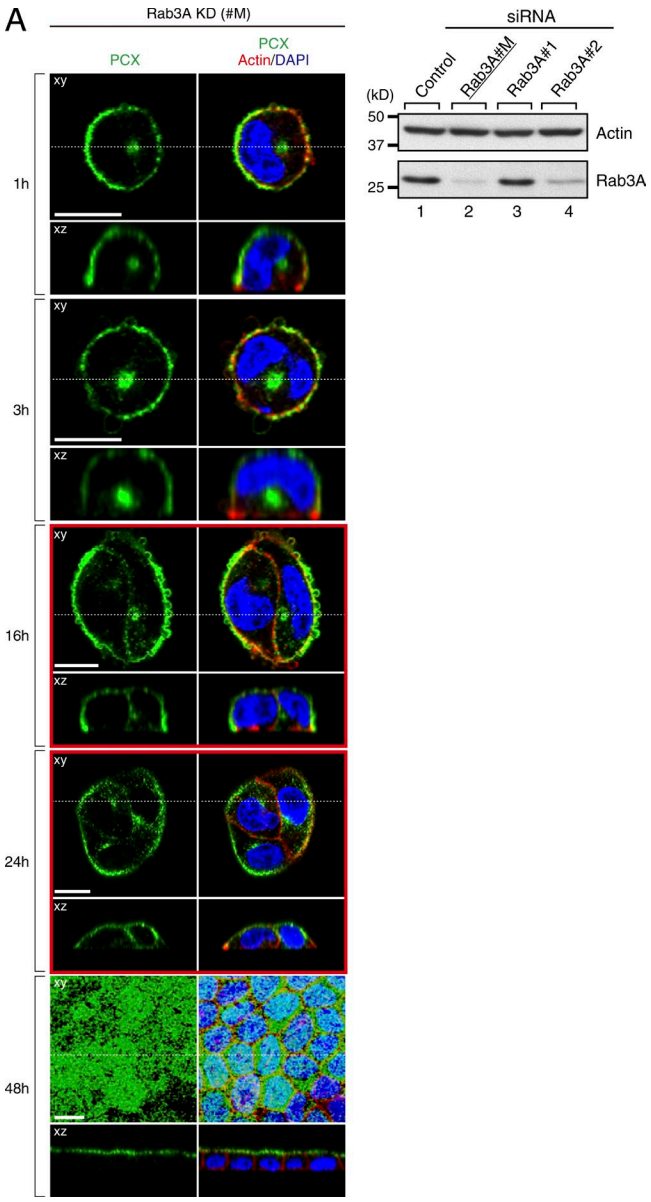

Figure S4. **Effect of KD of Rab GTPases on localization of endogenous PCX in 2D MDCK II cells.** (A–X) MDCK II cells that had been treated with indicated *Rab* siRNAs were plated on glass-bottom dishes and fixed with PFA at the times indicated (left). The cells were then stained with anti-PCX antibody (green), Texas red–conjugated phalloidin (red), and DAPI (blue). The confocal xy section (top) and the xz section (bottom), which corresponds to the dashed line in the xy section, are shown. Bars, 10 µm. Panels surrounded by a red box show MDCK II cells that exhibit defects in cell shape or PCX trafficking (summarized in Fig. 4). KD efficiency of *Rab* siRNAs as revealed by immunoblotting with specific antibody against each Rab or by RT-PCR (right). Except for *Rab12*, *Rab23*, and *Rab25* siRNAs, *Rab* siRNAs were transfected into MDCK II cells, and the level of endogenous Rab protein expression was detected with specific antibodies. Canine Rab23 and Rab25 were coexpressed with respective siRNAs in COS-7 cells to evaluate their KD efficiency. For Rab12, the level of its gene expression after siRNA treatment was determined by RT-PCR. The most effective siRNA (underlined) was used in this study. (Y) Immunoblot images showing expression levels of Rab GTPases, which were omitted in the KD screening because of lack of visible protein expression in MDCK II cells. Recombinant FLAG-tagged mouse Rabs were used as controls. No bands were detected in subconfluent (MDCK II 3 h; lane 2) and confluent (MDCK II 48 h; lane 3) MDCK II cells, although each antibody clearly recognized respective recombinant FLAG-Rab (lane 1) under the same experimental conditions. The positions of the molecular mass markers (in kilodaltons) are shown on the left. ns, nonspecific band. Panels B–X are not depicted here because of space limitations, but they are fully available in the JCB DataViewer at http://dx.doi.org/10.1083/jcb.201512024.dv.

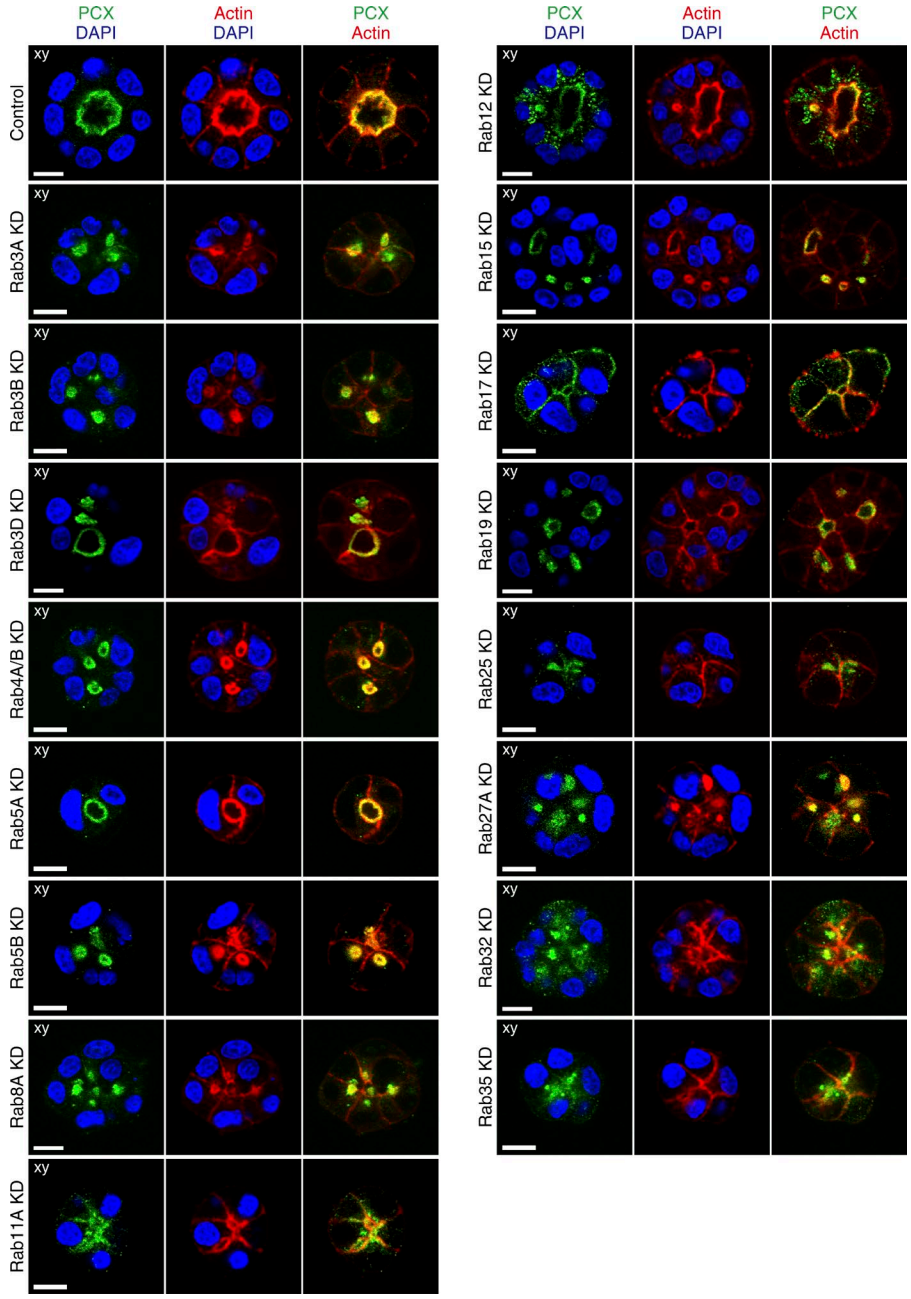

Figure S5. **Effect of KD of Rab GTPases on single-lumen formation in 3D MDCK II cells.** MDCK II cells that had been treated with control siRNA or indicated *Rab* siRNAs were plated on Matrigel-coated glass slides and fixed with PFA 72 h after plating. The cells were then stained with anti-PCX antibody (green), Texas red–conjugated phalloidin (red), and DAPI (blue). Representative microscopic images are shown. Percentage of single-lumen formation in cysts of Rab-KD cells (or control cells) was calculated, and the quantification results are shown in Fig. 5 A. Bars, 10 µm.

Table S1. **List of siRNA sequences used in this study**

Name	siRNA target sequence
ACAP2	5′-CCAGGATCATTCAGCCAAA-3′
Fascin	5′-GCAAGTTTGTGACAGCCAA-3′
MICAL1	5′-GCAGAGCGCTTTACACATT-3′
MICAL-L1	5′-GGACAATGTCTTCGAGAAT-3′
OCRL	5′-GGGAGTTTGTGTTTGAGAA-3′
PCX	5′-CCAGAGAGTTCTTCCGAAA-3′
Rab3A	5′-GGACAACATTAATGTCAAG-3′
Rab3B	5′-CCAATGAGGATTCCTTCAA-3′
Rab3D	5′-TGGGCTTCCTGCTGATGTA-3′
Rab4A	5′-TGCGCTTACTAATTGGTTA-3′
Rab4B	5′-GCCCCAACATCGTGGTCAT-3′
Rab5A	5′-GCACAGTCCTATGCAGATG-3′
Rab5B	5′-AGACAGCTATGAACGTGAA-3′
Rab5C	5′-GCCCCAGAATGCAGCTGGT-3′
Rab8A	5′-AGGCCAACATCAATGTGGA-3′
Rab10	5′-TGGGTATCATGCTAGTATA-3′
Rab11A	5′-GAAGCTGCTTTTCAGACAA-3′
Rab12	5′-TAGCATCCTTTCTCTACAA-3′
Rab13	5′-GAACGATTCAAGACAATAA-3′
Rab14	5′-GGCACAGAGAGATGTCACA-3′
Rab15	5′-CCATCACAAAGCAGTACTA-3′
Rab17	5′-GCGCTCTTGGTATACGACA-3′
Rab19	5′-CTTGGTCATTATGCTGATT-3′
Rab20	5′-GATCCTGAAGTACAAGATG-3′
Rab23	5′-CCAACAAACAGAGAACCAA-3′
Rab24	5′-AGGCTGCCATCGTCTGCTA-3′
Rab25	5′-CCAATGTAGAGCTGGCCTT-3′
Rab27A	5′-AGAGAGGTTTCGTAGCTTG-3′
Rab32	5′-TGACCCGAGTATACTACAA-3′
Rab34	5′-TGCATTGCGTCAACCTATT-3′
Rab35	5′-AGCGGTGGCTTCATGAAAT-3′