Kang and Fölsch, http://www.jcb.org/cgi/content/full/jcb.201012121/DC1

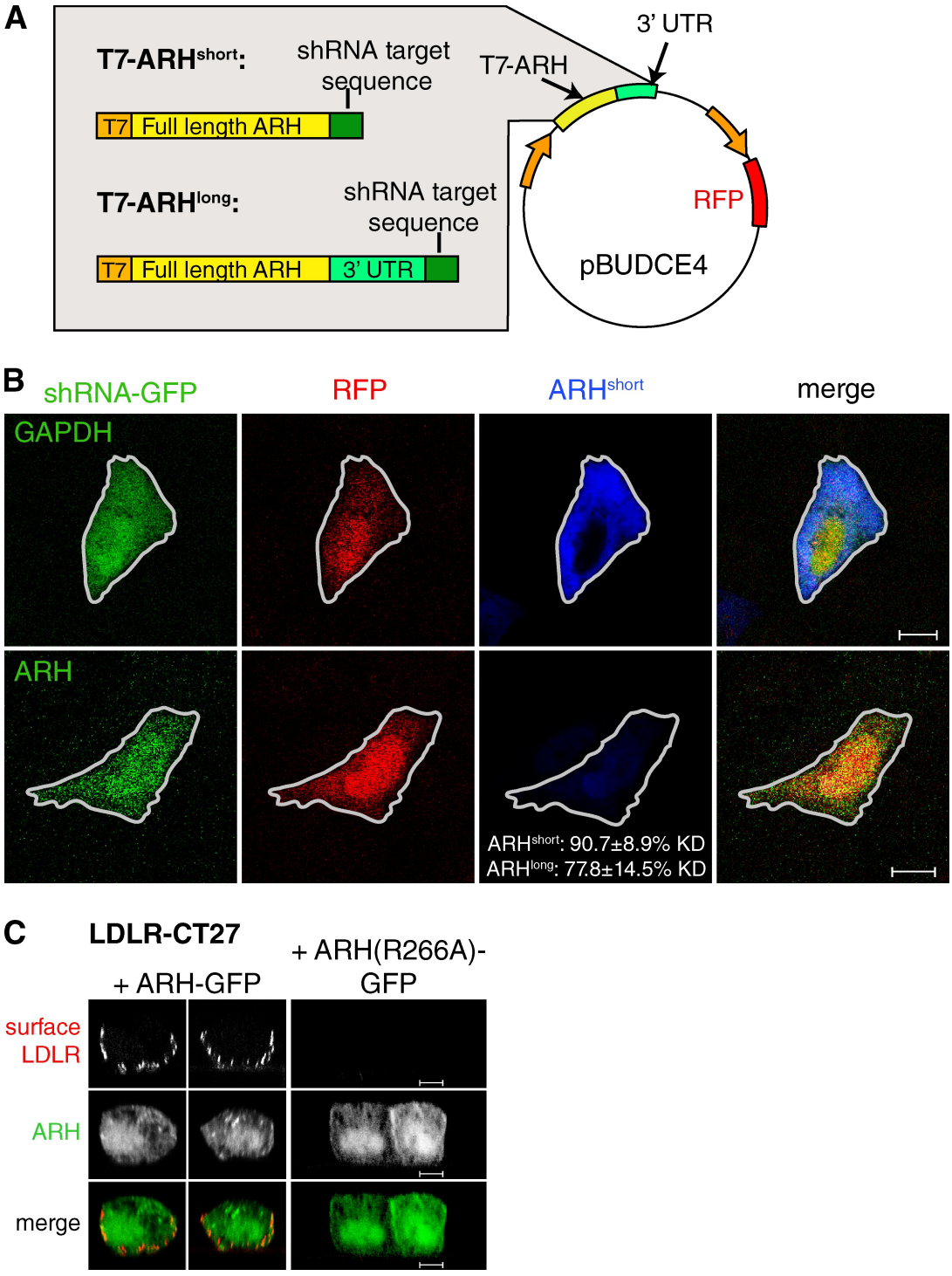
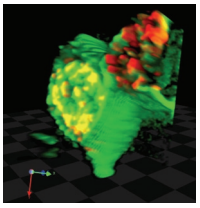


Figure S1. **Quantitative immunofluorescence analysis of ARH knockdown in individual HBE cells, and ARH-GFP overexpression in polarized MDCK cells.** (A) Schematic drawing of T7-tagged ARH with either short or long 3′ overhangs encoding the shRNA recognition sequence cloned into the bicistronic pBUDCE4 vector behind one promoter. DNA encoding RFP is cloned behind the second promoter. (B) HBE cells were seeded on coverslips and transiently transfected with plasmids encoding T7-ARH^{short} or T7-ARH^{long} together with plasmids encoding shRNAs targeting ARH or GAPDH. 24 h after transfection, cells were fixed and stained for T7-ARH. Shown are representative confocal images of HBE cells expressing T7-ARH^{short} and shRNAs targeting GAPDH (top) or ARH (bottom). At least 50 cells from at least three independent experiments for each condition were analyzed to determine mean values of ARH knockdown for T7-ARH^{short} and T7-ARH^{long} constructs. Errors are SD. Cells transfected with shRNA vectors are outlined in white. KD, knockdown. Bars, 10 µm. (C) Filter-grown MDCK cells were microinjected with cDNA encoding LDLR-CT27 together with cDNAs encoding ARH-GFP or ARH(R266A)-GFP. LDLR at the surface was stained with primary antibodies before fixation. Specimens were analyzed by confocal microscopy and representative images are shown. Bars, 5 µm.



Video 1. **ARH and TfnR colocalize in REs of polarized MDCK cells.** This QuickTime interactive movie shows a 3D reconstruction of a fully polarized MDCK cell infected with defective adenoviruses expressing ARH-GFP and stained for endogenous TfnR (in red).

Table S1. **Primers used for PCR, site-directed mutagenesis, RT-PCR, and qRT-PCR**

Oligo	Sequence
ARH-FL-N	5′-CGCG**GAATTC**ATGGACGCGCTCAAGTCGGCGGGG-3′
ARH-FL-C	5′-CGCG**TCTAGA**GAAGCTGAAGAGGTCATCCTGCTCTGTGC-3′
ARH-FL-C2	5′-CGCG**AAGCTT**TCAGAAGCTGAAGAGGTCATCCTGCTCTGTGC-3′
ARH-FL-V5-C	5′-CGCG**AAGCTT**TCATGTGCTGTCTAATCCTAATAAGGGGTTTGGTATTGGCTTTCCGAAGCTGAAGAGGTCATCCTGCTCTGTGC-3′
ARH-T7-N	5′-CGCG**GTCGAC**ATGGCTAGCATGACTGGTGGACAGCAAATGGGTGACGCGCTCAAGTCGGCGGG-3′
ARH-UTR1-C	5′-CGCG**TCTAGA**TTAATGCTTTAAAGTGCCAAGCTGTCAGAAGCTGAAGAGGTCATCCTGCTCTGTGC-3′
ARH-UTR2-C	5′-CGCG**TCTAGA**CCCCTTCTCCTGGAGGAAGGGCAGAAG-3′
ARH(R266A)-sense	5′-CGAGGCTTGCCCAGTCTGCGACAAACCCTCAGGTC-3′
RT ARH-sense	5′-CGCG**GAATTC**ATGGACGCGCTCAAGTC-3′
RT ARH-anti	5′-CGCG**GGATCC**TCAGAAGCTGAAGAGGTCATCC-3′
RT Rab8-sense	5′-GCGC**GAATTC**ATGGCGAAGACCTACGATTAC-3′
RT Rab8-anti	5′-GCGC**GGATCC**TCATCGGAAAAAGCTGCTCCTCTT-3′
RT Rab10-sense	5′-GC**AGATCT**ATGTACCCATACGACGTCCCAGACTACGCCGCGAAGAAGACGTACGACCTGCTTTTC-3′
RT Rab10-anti	5′-GCGC**AAGCTT**TCAGCAACATTTGCTCTTCCAGCC-3′
qRT GAPDH-sense	5′-GCCAAGAGGGTCATCATCTC-3′
qRT GAPDH-anti	5′-AGGAGGCATTGCTGACAATC-3′
qRT ARH-sense	5′-TTGCCTACATTGCACAGAGC-3′
qRT ARH-anti	5′-CTTGGACACCTGCCAAAACT-3′
qRT Rab8-sense	5′-CACTCTCGCCAGAGACATCA-3′
qRT Rab8-anti	5′-AAGCTGCTCCTCTTCTGCTG-3′
qRT μ1B-sense	5′-TGGGGTCAAGTTTGAGATCC-3′
qRT μ1B-anti	5′-GCCCGGTAACCTCTTTTCTC-3′

Restriction sites are indicated with bold letters, and mismatched sequences for site-directed mutagenesis are underlined. RT, primers used for RT-PCR; qRT, primers used for qRT-PCR.