Supplemental Data

HIV-1 requires Arf6-mediated membrane dynamics to efficiently enter and infect T Lymphocytes

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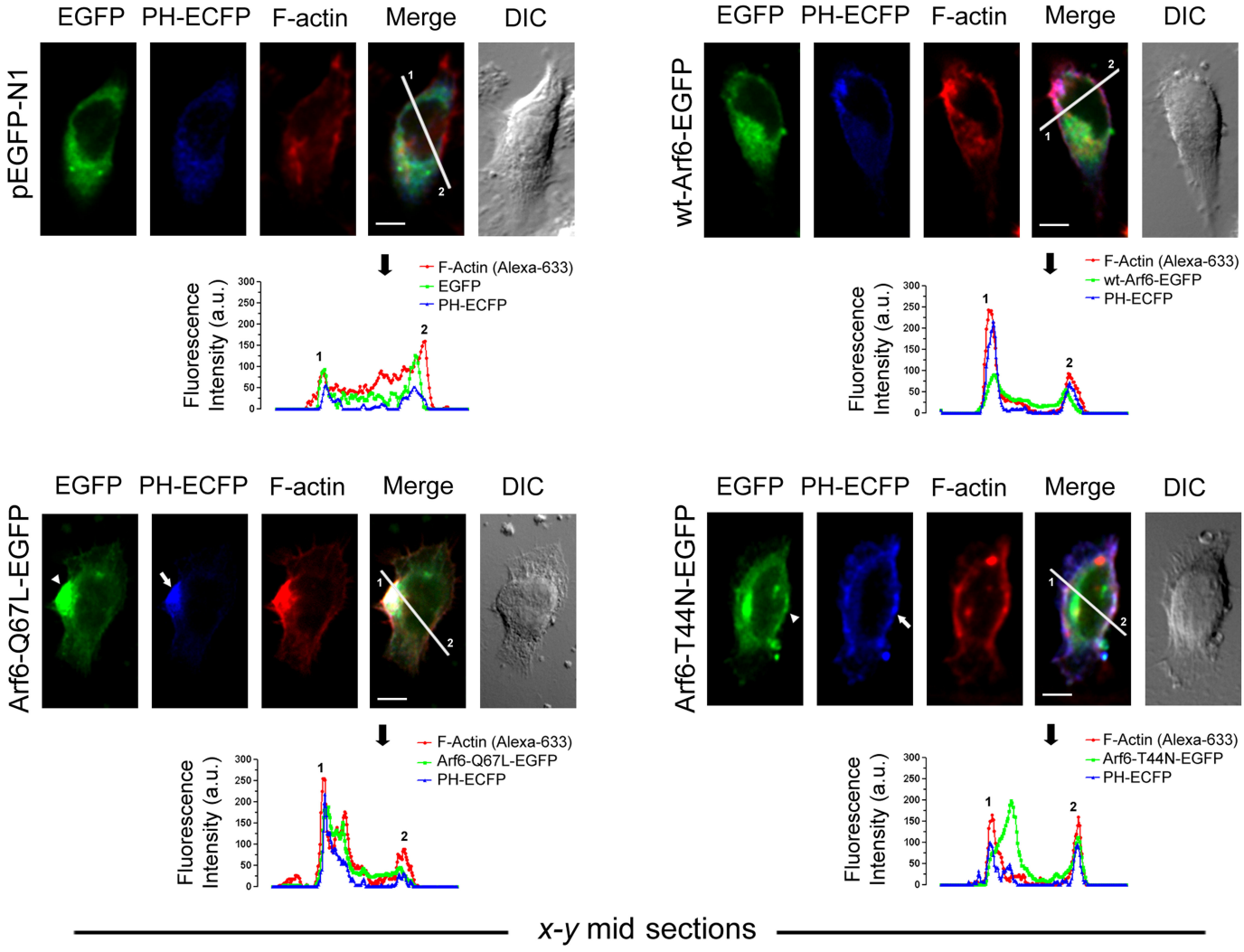

Figure S1. Expression pattern of Arf6-EGFP constructs on permissive HeLa cells. A series of confocal images, x-y mid sections, show the expression pattern for wt-Arf6-, Arf6-Q67L- and Arf6-T44N-EGFP constructs in TZMbl cells. pEGFP-N1 plasmid is a control for free EGFP expression. PIP_2 (PH-ECFP) and F-actin (Alexa 633-labelled phalloïdin) distribution, and Merge and DIC images are shown. The quantification of the pattern of co-distribution of each EGFP-labelled Arf6 construct or the free EGFP protein with the PH-ECFP probe or F-actin is shown by line scan quantification. Bar, 5 μm. A representative experiment of three is shown.

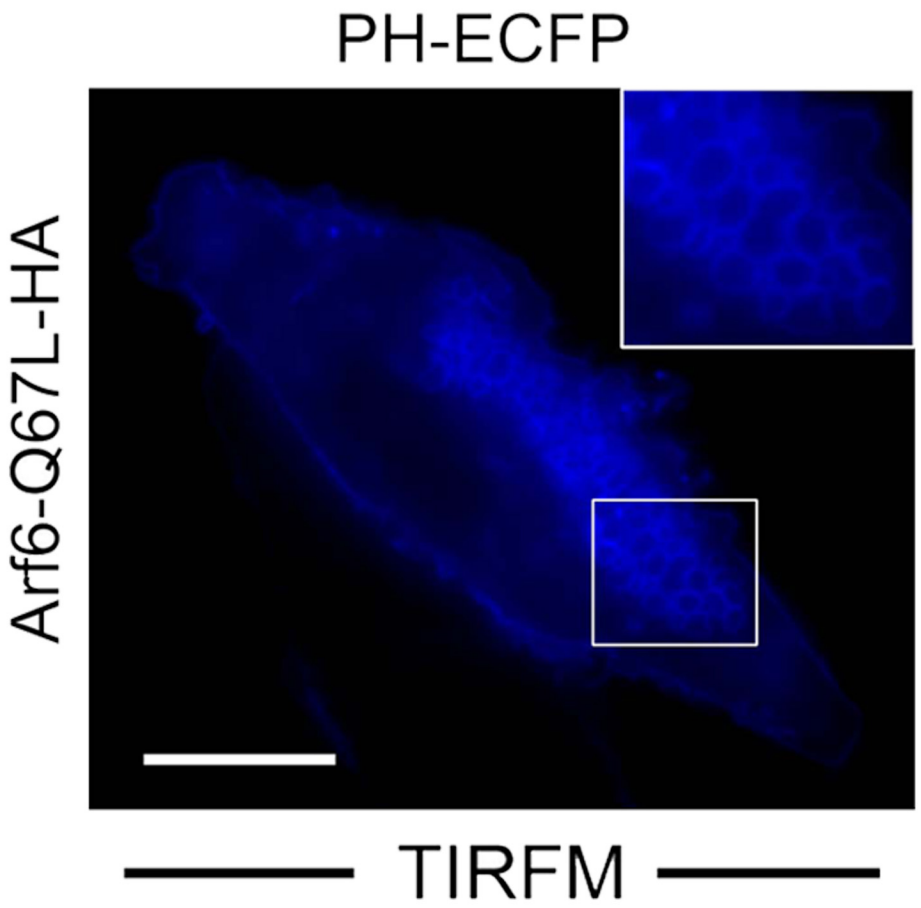

Figure S2. Accumulation of PIP_2-associated vacuole-like structures near the plasma membrane provoked by the Arf6-Q67L-HA mutant.
TIRFM analysis of PIP_2-associated vacuole-like structures accumulated at the EF of permissive TZMbl cells, transiently transfected with the Arf6-Q67L-HA mutant.
Top right square shows a zoom from the region indicated at the plasma membrane of the entire cell. Bar, 5μm.

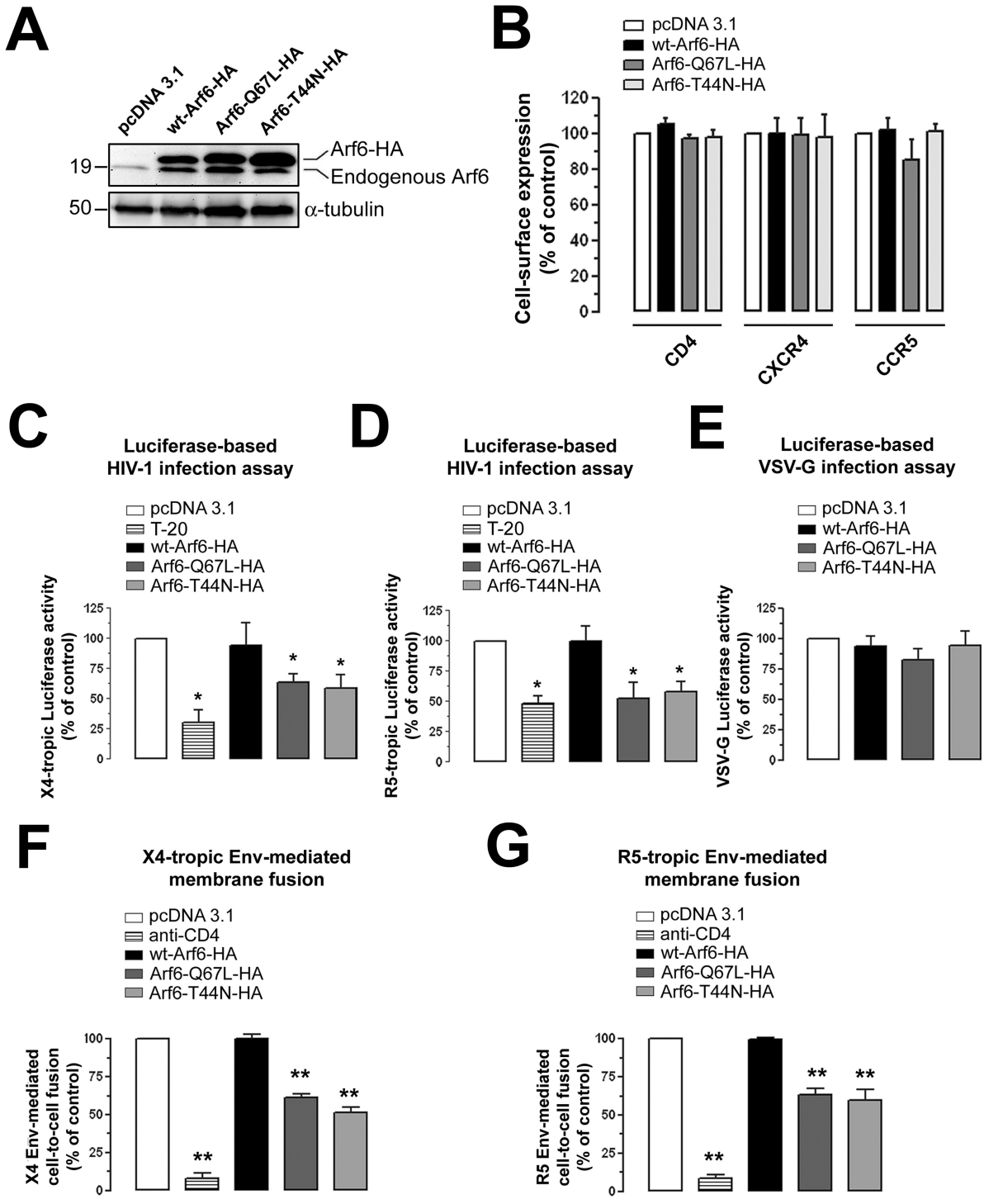

Figure S3. Effect of Arf6-HA mutants on HIV-1 Env-mediated entry and infection, and membrane fusion in permissive HeLa cells. (A) Western-blot analysis of endogenous Arf6, wt-Arf6-, Arf6-Q67L-, and Arf6-T44N-HA expression in HeLa-P5 cells. α-tubulin and pcDNA3.1 are the controls for total protein and transfected cells, respectively. A representative experiment of three is shown. (B) Flow cytometry analysis of CD4, CXCR4 and CCR5 cell-surface expression in Arf6-HA-transfected HeLa-P5 cells. Data are mean ± SEM, n = 9. (C and D) Luciferase-based assay of HIV-1 entry and infection by non-replicative X4- and R5-tropic viruses, respectively, in Arf6-HA-transfected HeLa-P5 cells (control, 100% viral entry and infection in pcDNA3.1-transfected cells). When indicated, viral infection was inhibited by the anti-fusogenic T-20 peptide in cells transfected with pcDNA3.1. Data are mean ± SEM of three independent experiments carried out in triplicate. Asterisk indicates $p < 0.05$, *t* test. (E) VSV-G virions from Luciferase HIV-1 vectors were used to control the specificity of Arf6 mutants-mediated effects on HIV-1 viral entry and infection (control, 100% viral entry and infection in pcDNA3.1-transfected cells). Values are mean ± SEM, n=9. (F and G) Arf6-HA constructs effect on HIV-1 Env-mediated membrane fusion by X4- and R5-tropic Env, respectively (control, 100% HIV-1-Env-mediated membrane fusion with pcDNA3.1-transfected Env(-) cells). When indicated, cell-to-cell fusion was inhibited by a neutralizing anti-CD4 mAb and using pcDNA3.1-transfected Env(-) cells.
Values are mean ± SEM, n=9. Asterisks indicates $p < 0.01$, *t* test.

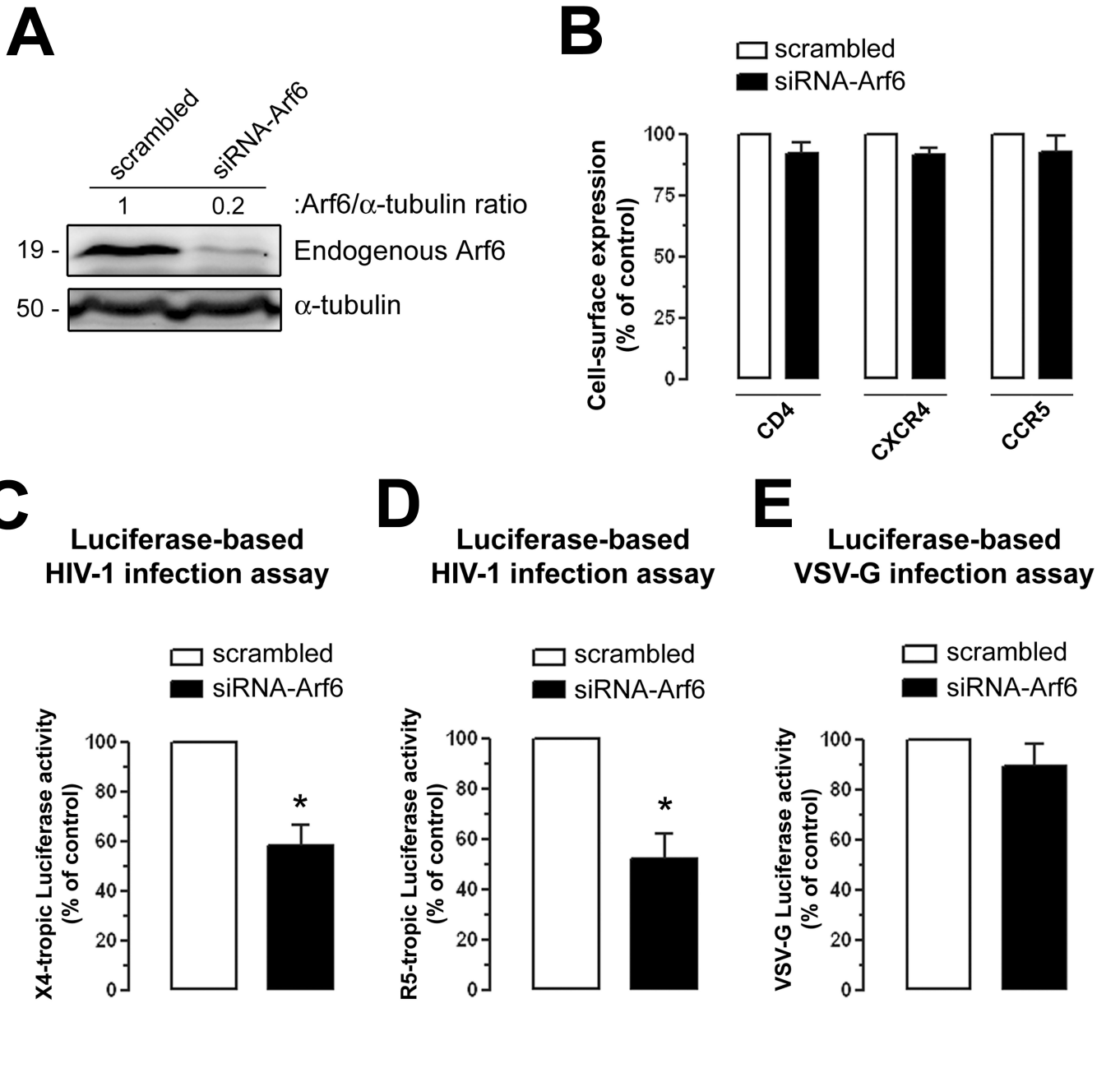

Figure S4. Effect of specific Arf6 silencing on HIV-1 entry and infection in permissive HeLa cells. (A) Western-blot analysis of endogenous Arf6 knock-down in HeLa-P5 cells quantified as the band intensity ratios to α-tubulin. (B) Flow cytometry analysis of CD4, CXCR4 and CCR5 cell-surface expression in Arf6-silenced cells. Data are the mean ± SEM of three independent experiments carried out in triplicate. (C and D) Luciferase-based assay of viral entry and infection by with X4- and R5-tropic virus in Arf6-silenced HeLa-P5 cells. Control is defined as 100% viral entry in cells transfected with scrambled oligonucleotides. Data are mean ± SEM of three independent experiments carried out in triplicate. Asterisk indicates $p < 0.05$, *t* test. (E) VSV-G virions from Luciferase HIV-1 vectors were used to control the specificity of Arf6 knock-down-mediated effects on HIV-1 viral entry and infection (control, defined as 100% viral entry and infection in scrambled-transfected cells).
Values are mean ± SEM, n=9.

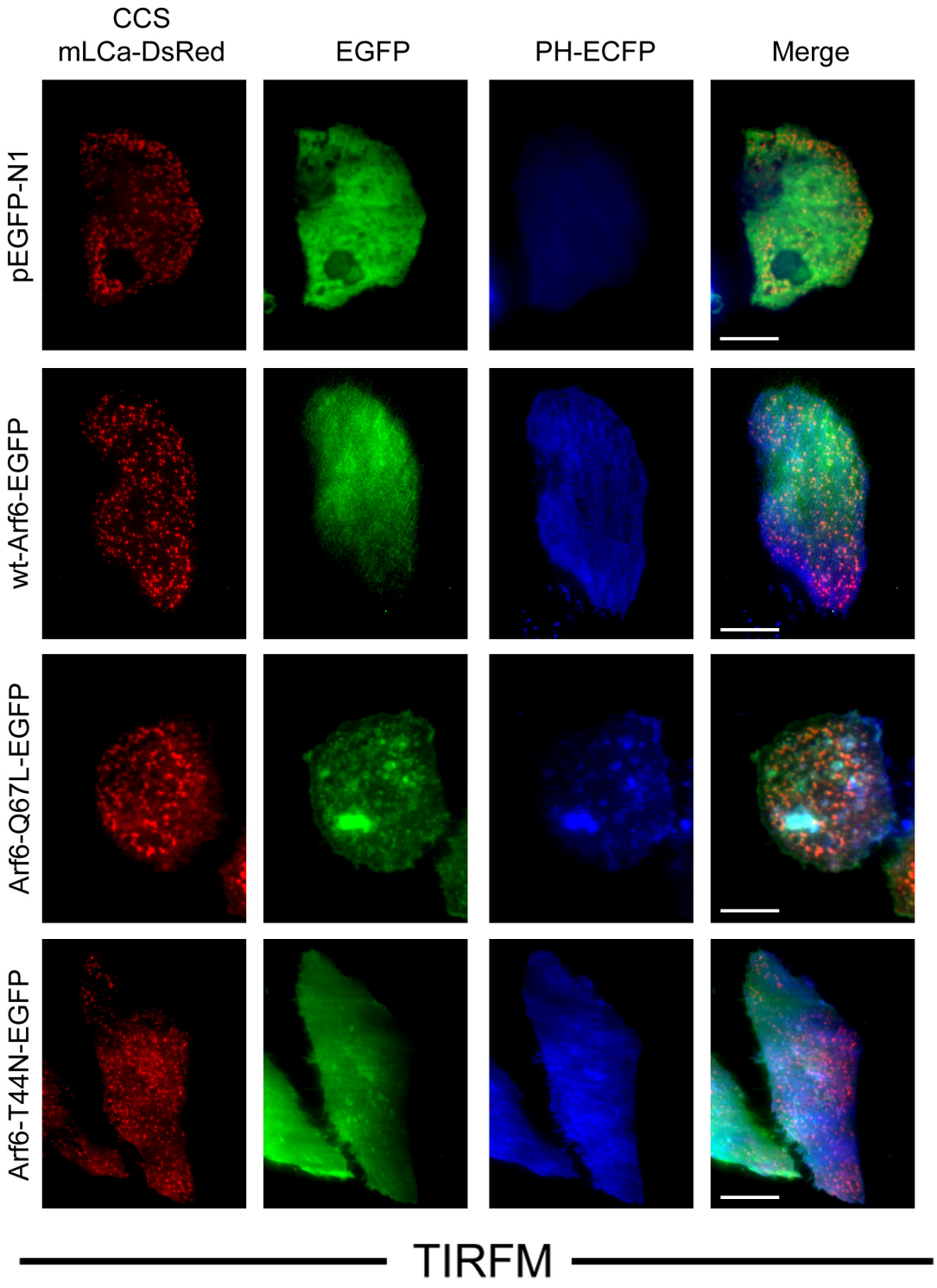

Figure S5. Clathrin-coated structures (CCS) at the plasma membrane of permissive cells transfected with the different Arf6-EGFP constructs. A series of TIRFM images showing the expression pattern of clathrin-coated structures (CCS), monitored by fluorescent mLCa-DsRed, at the plasma membrane of permissive TZMbl cells, transiently transfected with each Arf6-EGFP construct. Distribution of Arf6-EGFP constructs and PH-ECFP probe (read-out for PIP_2) at the EF of cells are shown. Merge images are shown. Bar, 5μm.
A representative experiment of three is shown.