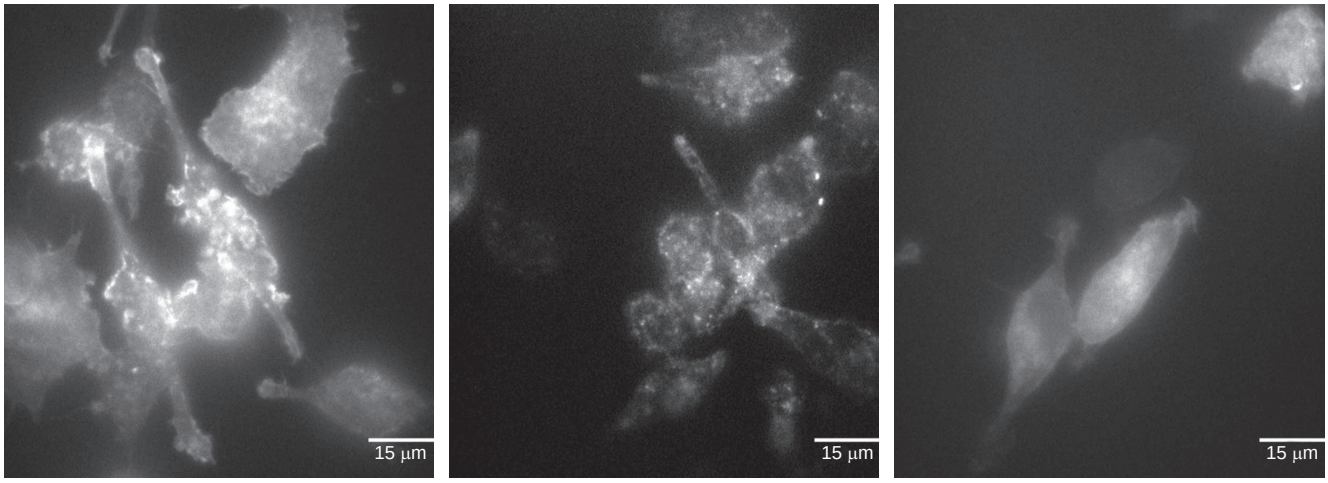
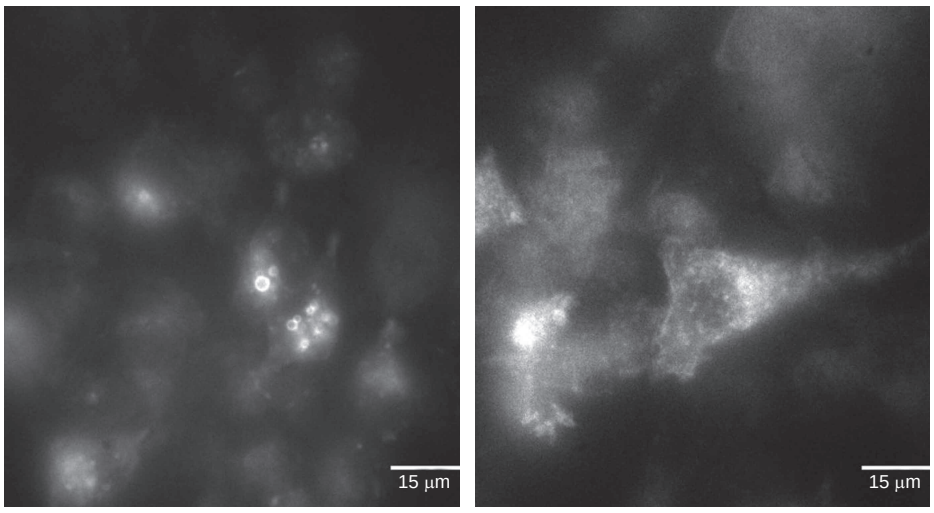
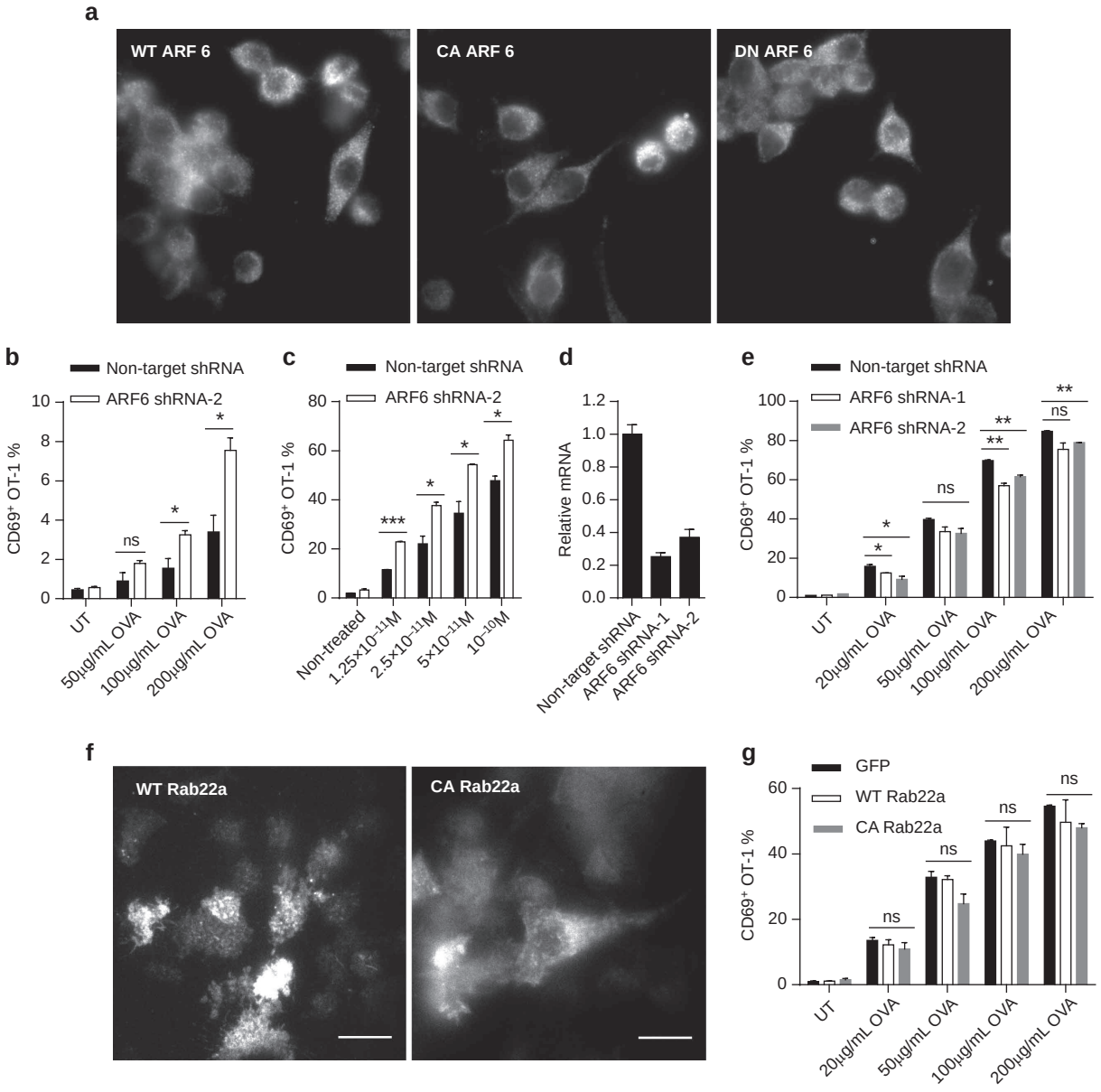


Movies 1, 2 and 3 In DC2.4 cells overexpressing WT, ARF6 resided predominantly at the plasma membrane and some endosomal compartments (Movie 1). Cells overexpressing CA mutant ARF6-Q67L (defective in GTP hydrolysis) showed enlarged endosomal structures, characteristic of a blockade in transport of ARF6 cargo on the way to early endosomes (Movie 2). In contrast, these enlarged endosomal structures were not observed in cells expressing DN mutant ARF6-T27N (defective in GTP binding, Movie 3). It has been reported that ARF6-T27N localized exclusively to tubulovesicular structures in Hela cells. We observed some ARF6-T27N compartments in cytoplasm, but the tubulovesicular structures were not as obvious as reported in Hela, possibly because of differences in cell types or limited microscopy resolution.



Movies 4 and 5 Identical to movies 1, 2 and 3 except that mCherry-tagged WT and CA mutant of Rab22a were used for transfection in place of ARF6 mutants.



Supplemental Figure 1 (**a**) DC2.4 cells overexpressing C-terminal mCherry-tagged WT, CA or DN ARF6 were fixed and probed with anti-H-2Kb antibody followed by the Alexa 488 Donkey Anti-Mouse IgG. (**b**) Identical to Figure 1e except that DCs were fixed with PFA prior to the cross-presentation assay. (**c**) DC2.4 cells expressing non-target or ARF6 shRNA were stimulated with SIINFEKL at the indicated concentrations for 4 h and then the fixed DC2.4 cells were cocultured with OT-1 cells for 24 h. (**d**) shRNA ARF6 knockdown efficiency in DC1940 cells. (**e**) Identical to Figure 1e, except that DC1940 cells were used in place of DC2.4 cells. (**f**) WT or CA Rab22a-overexpressing DC2.4 cells were processed for live imaging as in Figure 1a. (**g**) WT and CA Rab22a overexpressing cells were analyzed for the crosspresentation as in Figure 1c.