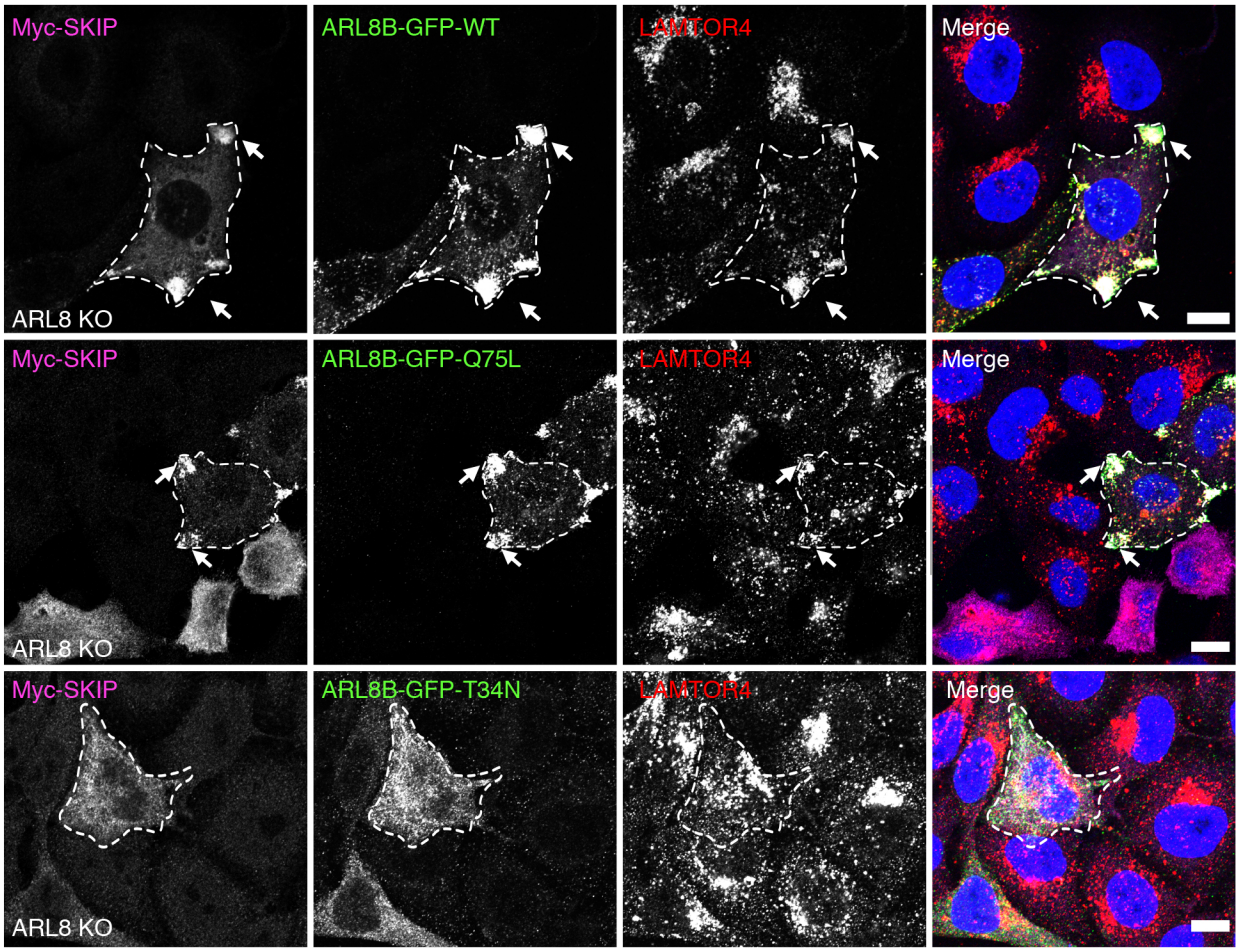

Figure S1. Effect of ARL8 mutants on the function of SKIP. Related to Figure 3. Confocal immunofluorescence microscopy of ARL8-KO HeLa cells transfected with plasmids encoding WT, Q75L (GTP-bound) or T34N (GDP-bound) forms of ARL8B-GFP. Cells were immunostained with antibodies to the Myc epitope (magenta) and endogenous LAMTOR4 (red), and counterstained with DAPI (blue). ARL8B-GFP was directly visualized by GFP fluorescence (green). Single channels are shown in grayscale. Cell edges are outlined. Scale bars: 10 µm. Notice that expression of ARL8-GFP-WT or ARL8-GFP-Q75L, but not ARL8-GFP-T34N, enable redistribution of Myc-SKIP and lysosomes to cell vertices (arrows).

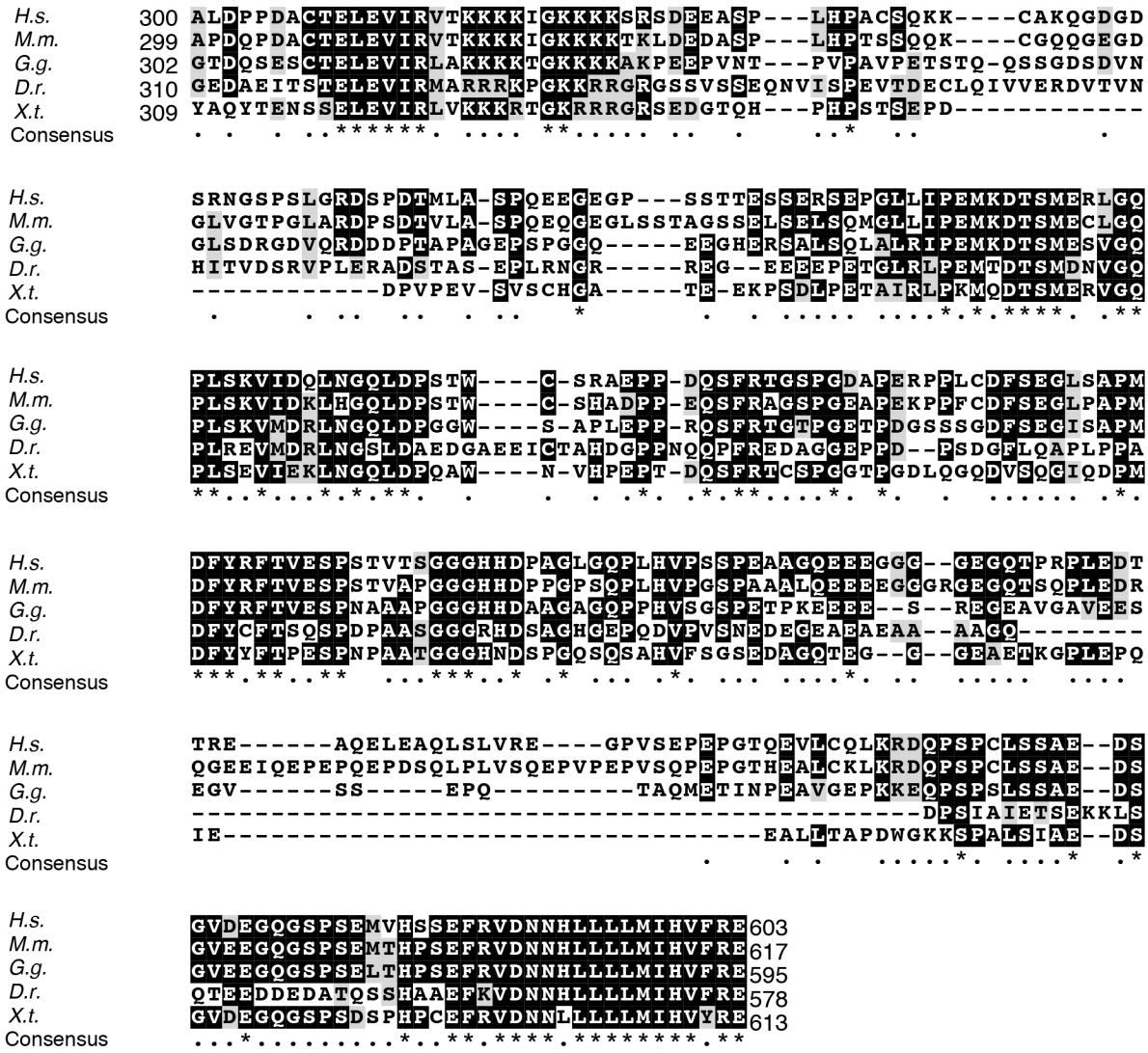

Figure S2. Multiple sequence alignment of human SKIP$_{300\text{-}603}$ with homologous regions of SKIP from different species. Related to Figure 4. Alignment was performed using the T-COFFEE server (expresso mode) (http://tcoffee.crg.cat). The figure was generated using BOXSHADE (https://embnet.vital-it.ch/software/BOX_form.html). Abbreviations: *H.s., Homo sapiens; M.m.*, *Mus musculus*; *G.g.*, *Gallus gallus*; *D.r., Danio rerio*; *X.t.*, *Xenopus tropicalis*. Conserved residues (*) are highlighted with black boxes and semi-conserved residues (.) are highlighted with gray boxes.

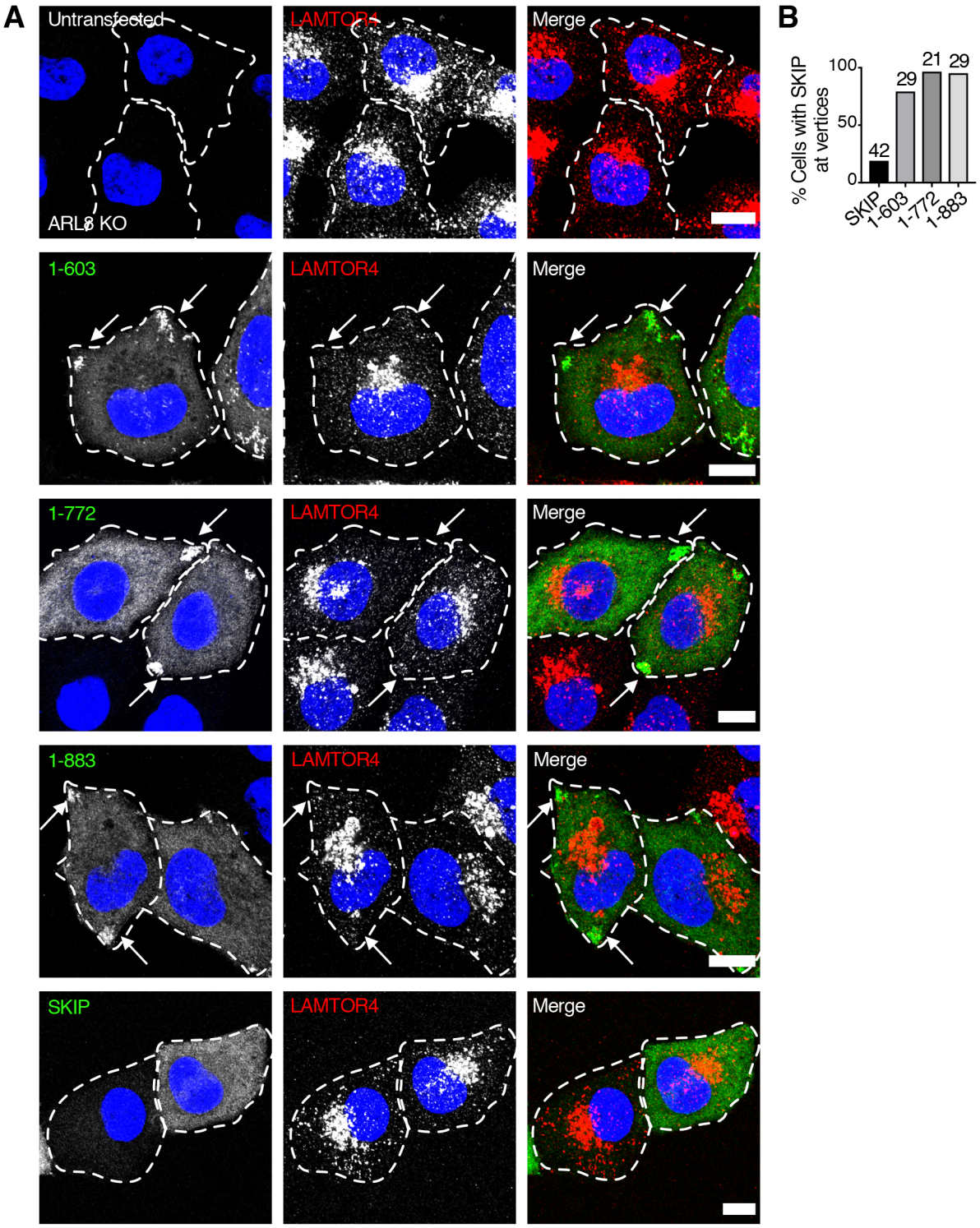

Figure S3. Analysis of the effect of SKIP deletion mutants in ARL8-KO cells. Related to Figure 5. (A) Confocal immunofluorescence microscopy of full-length and C-terminally truncated forms of Myc-SKIP (Figure 2A) expressed by transfection in ARL8-KO cells and immunostained with antibodies to the Myc epitope (green) and the lysosomal marker LAMTOR4 (red). Nuclei were stained with DAPI (blue). Single channels are shown in grayscale. Cell edges are outlined. Scale bars: 10 µm. (B) Quantification of the percentage of cells exhibiting SKIP accumulation at cell vertices. Numbers of cells used in the calculations for each condition are indicated on top of each bar. Notice that all C-terminally truncated constructs of Myc-SKIP, but not full-length SKIP, are associated with cell vertices in ARL8-KO cells. In contrast, lysosomes remain clustered in the center of these cells because of the absence of ARL8.