



VIRAL RECEPTOR TRAFFICKING REGULATED BY GABARAP

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Introduction

Human cytomegalovirus (HCMV) is a widespread pathogen that can lay dormant and rarely causes disease in healthy individuals. However, in immune-compromised individuals, such as transplant recipients and AIDS patients, HCMV infection can be life-threatening.

In this study, we examined one viral gene, US27, which encodes a protein that is similar to human chemokine receptors. Chemokine receptors have seven transmembrane domains and signal immune cells to sites of infection. Previous work in our lab found that US27 binds to GABARAP (γ -aminobutyric acid receptor-associated protein), a 14kD cellular protein that plays a role in receptor trafficking.

We used fluorescence microscopy to examine the US27-GABARAP interaction in human embryonic kidney cells (HEK293). GABARAP has been shown to bind to a WXXL motif in target proteins, and this sequence is present in the C-terminal domain of US27. Using a series of proteins with mutations in this motif (Figure 1), we found that the WXXL motif in US27 is required for both GABARAP binding and for localization to the endosomes.

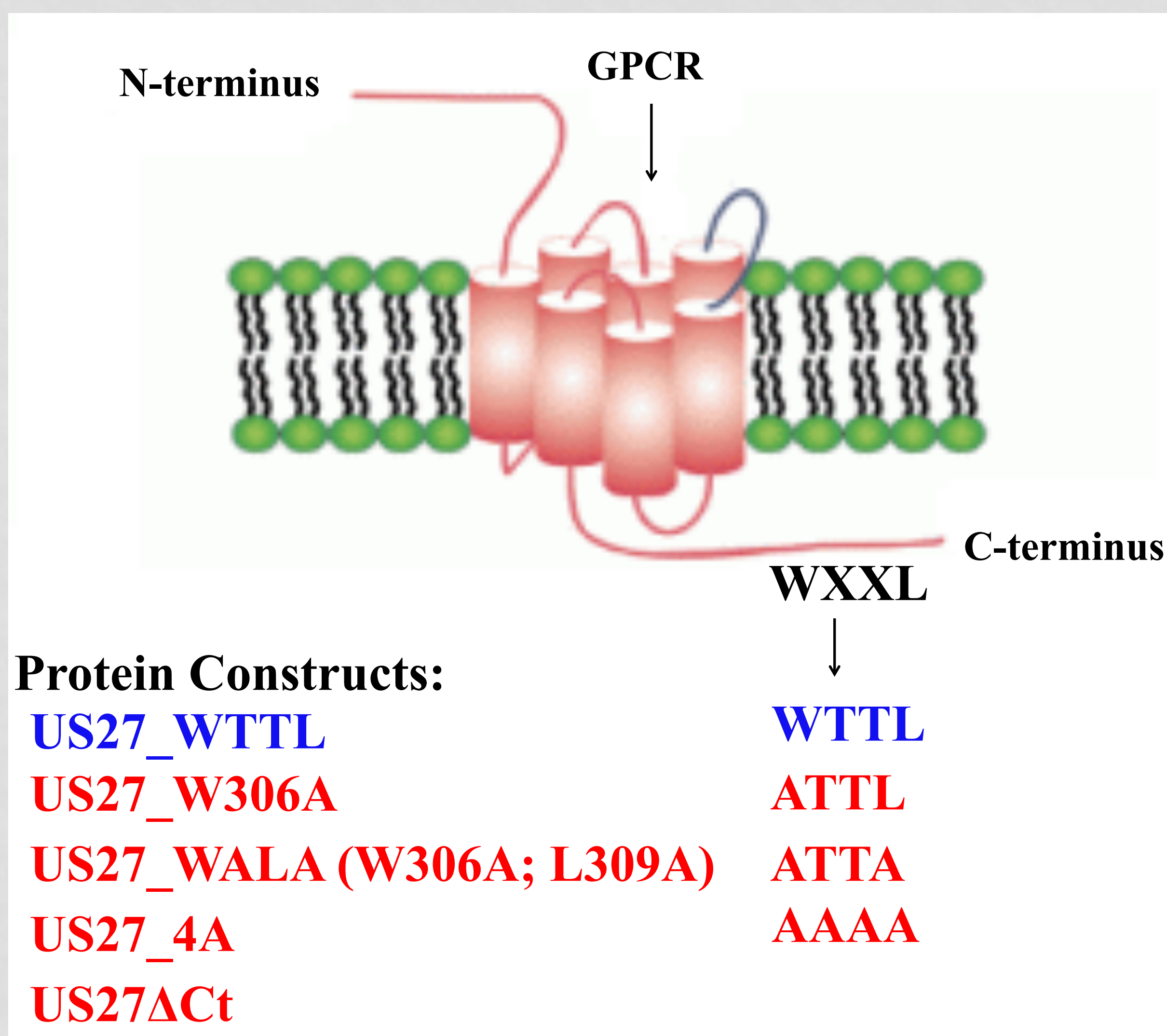


Figure 1. Schematic diagram of the US27 protein. Amino acid sequences in the C-terminal domain are shown with wild-type US27 in blue and mutants in red.

Results

US27 co-localizes with GABARAP; US27 mutants do not

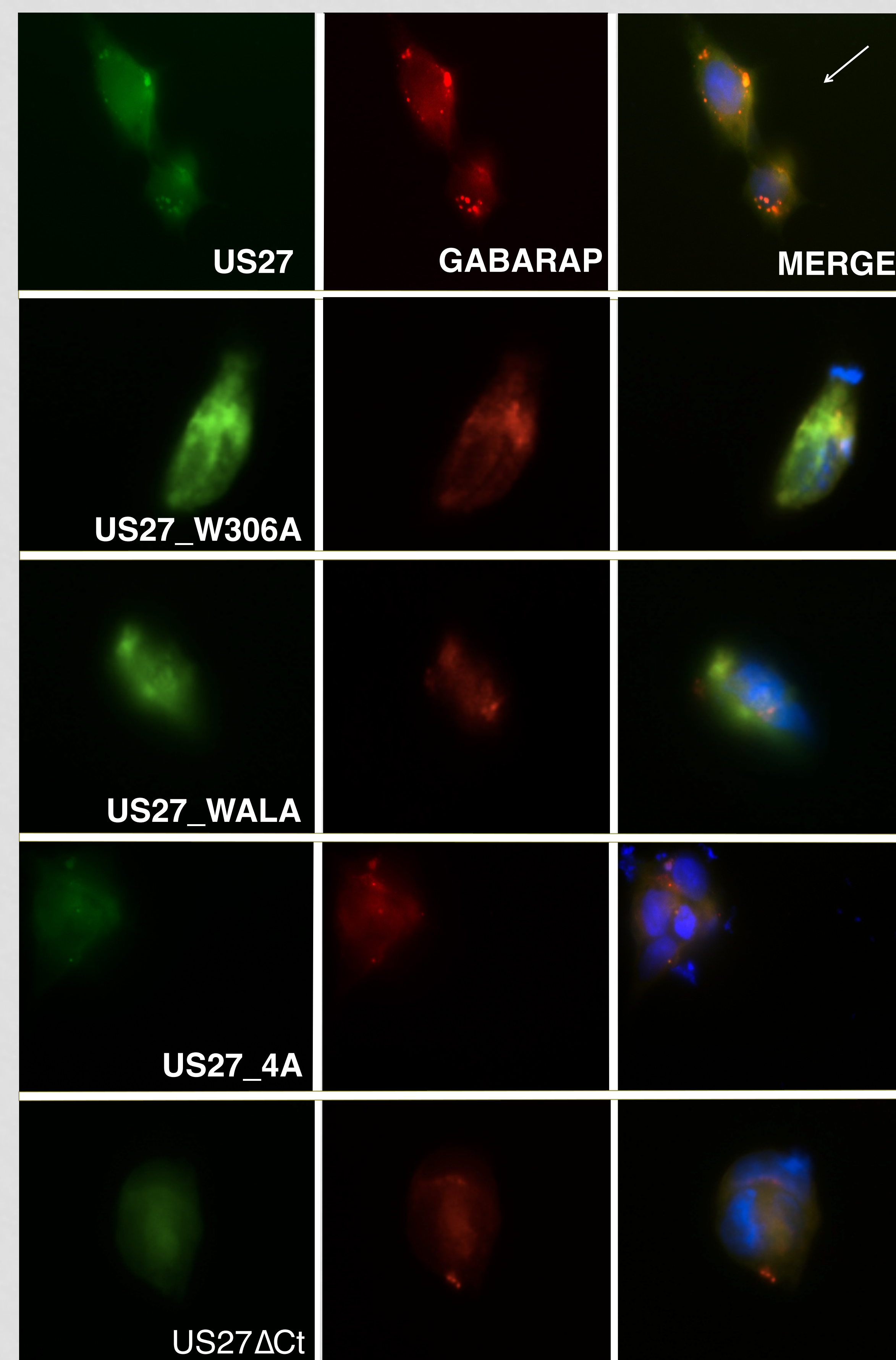


Figure 2. Fluorescence microscopy of US27 and mutants with GABARAP in human cells. The US27 gene was cloned into the pEGFP vector to create a US27-EGFP fusion protein (green). The GABARAP gene was cloned into the pDsRed vector to create a GABARAP-DsRed fusion protein (red). HEK293 cells were seeded onto coverslips and transfected with both vectors. After 48 hours, cells were fixed and mounted using Prolong Gold with DAPI to stain nuclei blue. In the merged image, areas of yellow represent co-localization (arrow).

US27 localizes to the endosomes; US27 mutants do not

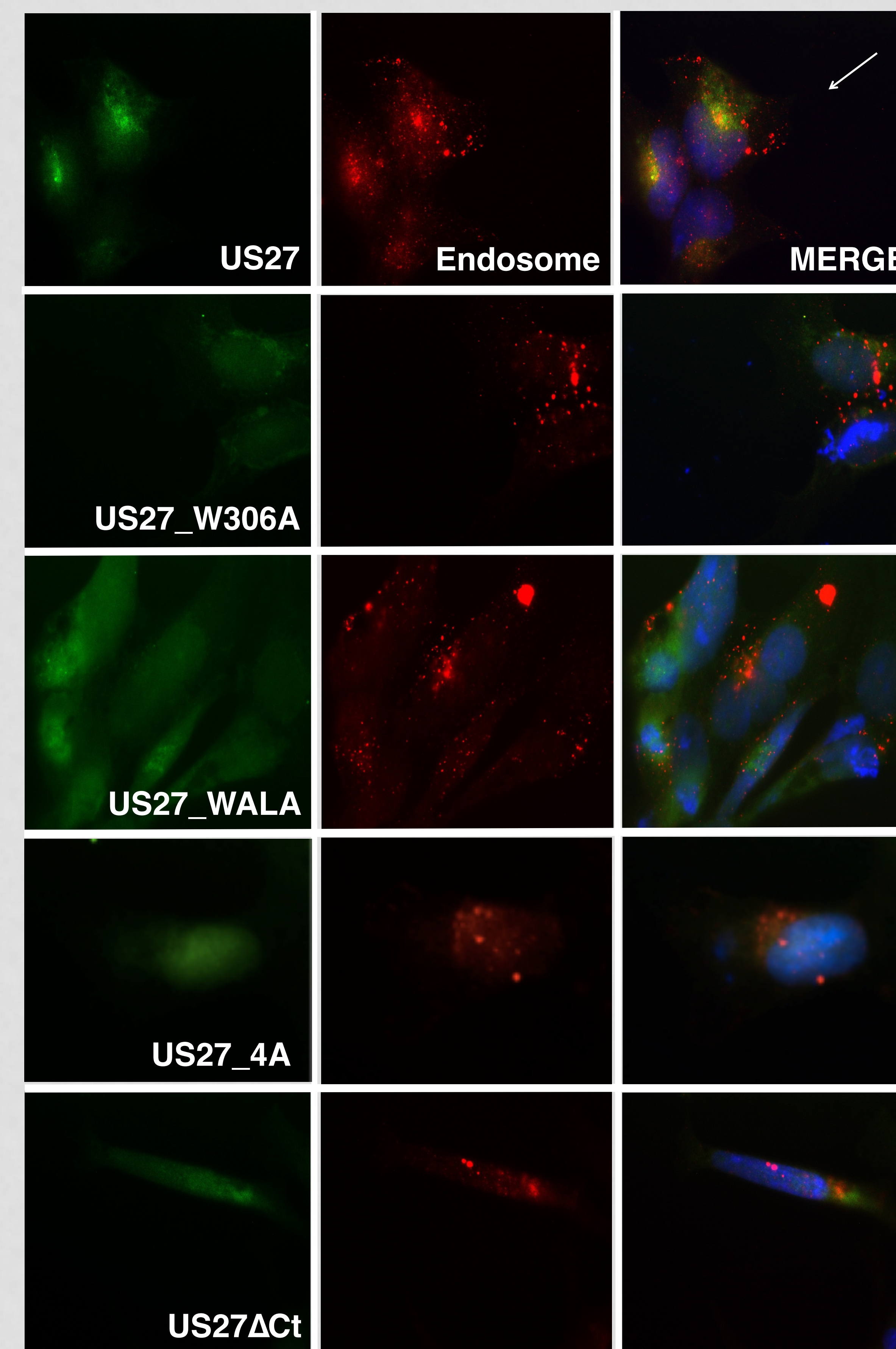


Figure 3. Immunofluorescence images showing wild-type US27 localized to the endosomes. HEK293 cells were seeded onto coverslips and transfected with GFP fusion vectors. After 48 hours, cells were fixed and stained with antibodies to EEA1 (early endosome antigen 1) followed by TRITC-conjugated goat anti-rabbit antibody (red). Blue represents the DAPI-stained nucleus, and green represents US27. In the merged image, areas of yellow represent co-localization (arrow).

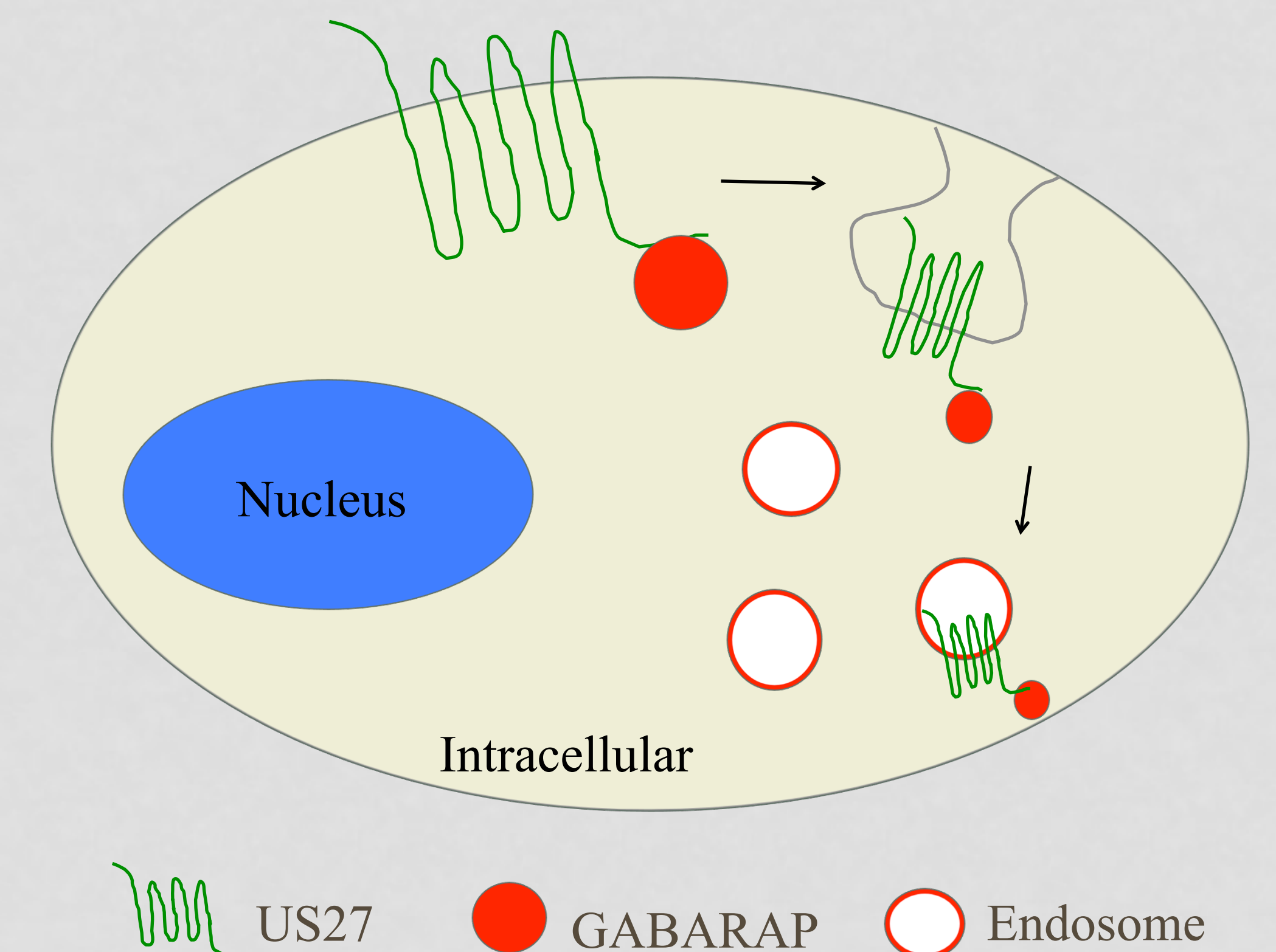


Figure 4. Cartoon drawing of the US27 receptor binding to GABARAP and localizing to the endosomes inside a cell.

Conclusions

- Fluorescence microscopy showed that the interaction of US27 and GABARAP requires a WXXL motif found in the C-terminal domain of US27.
- Mutation of the tryptophan (position 306) and leucine (position 309) residues in the C-tail of US27 disrupts its binding to GABARAP and localization to the endosomes. The interaction of wild-type US27 with GABARAP is depicted in Figure 4.
- These results show that US27 functions primarily as an intracellular protein. Our findings suggest that the viral receptor could induce internalization of cellular chemokine receptors and may modulate host chemokine signaling responses.
- Future work includes identifying additional proteins that are part of the US27-GABARAP complex with the aim of clarifying the role of US27 during virus infection.

Acknowledgements

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