

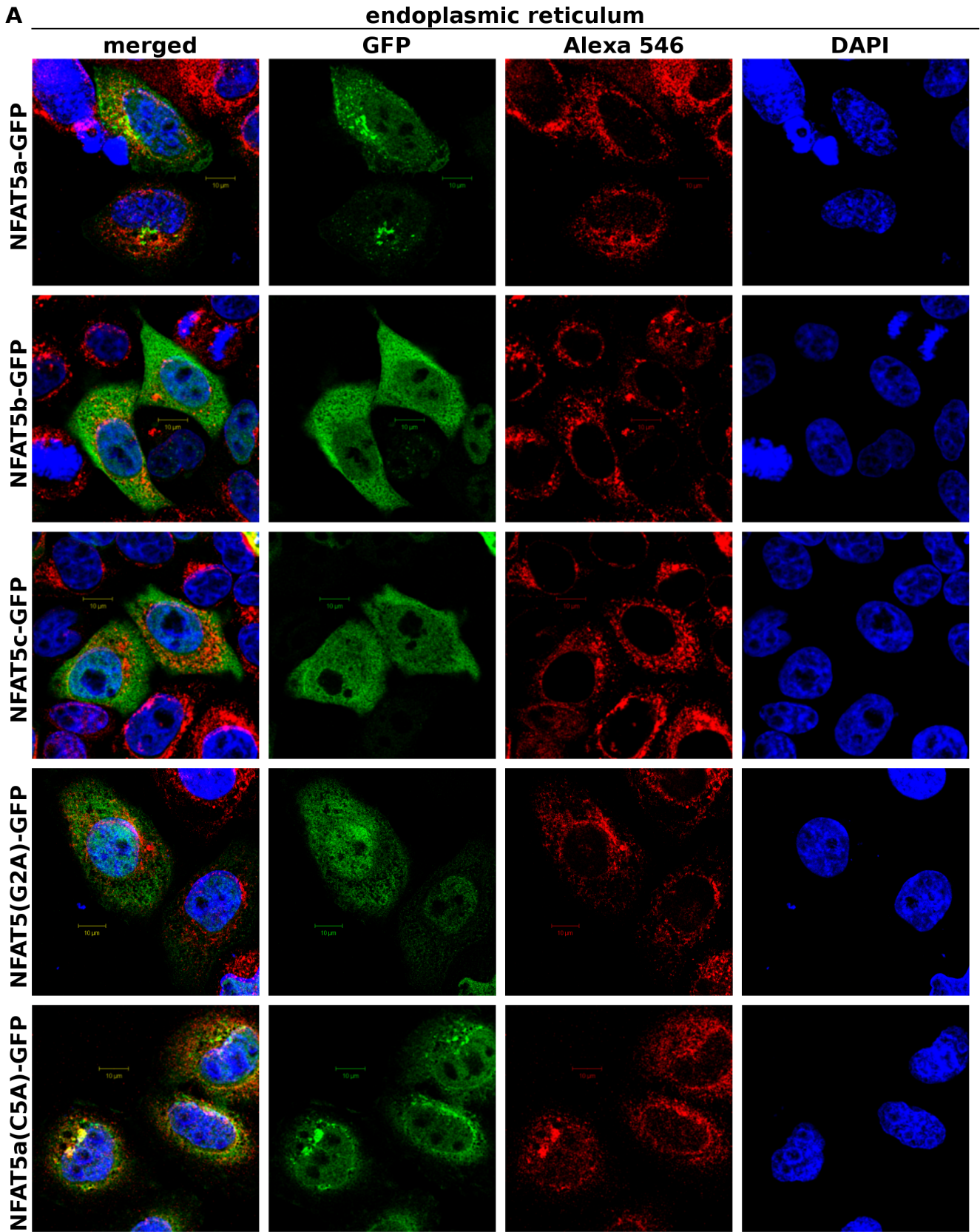
Full-size images for Figure 3

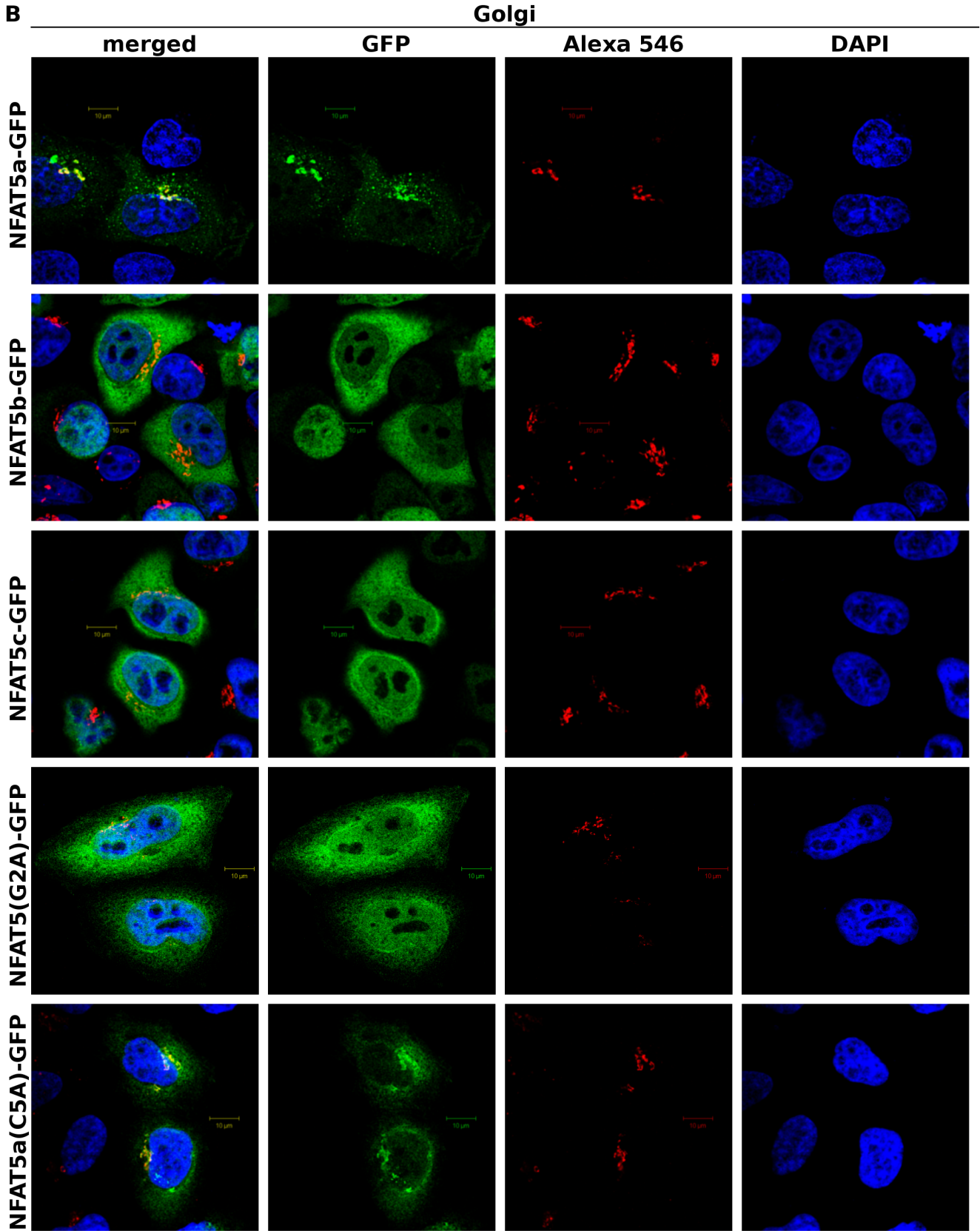
Localization studies of NFAT5a/b/c and its mutants in HeLa cells

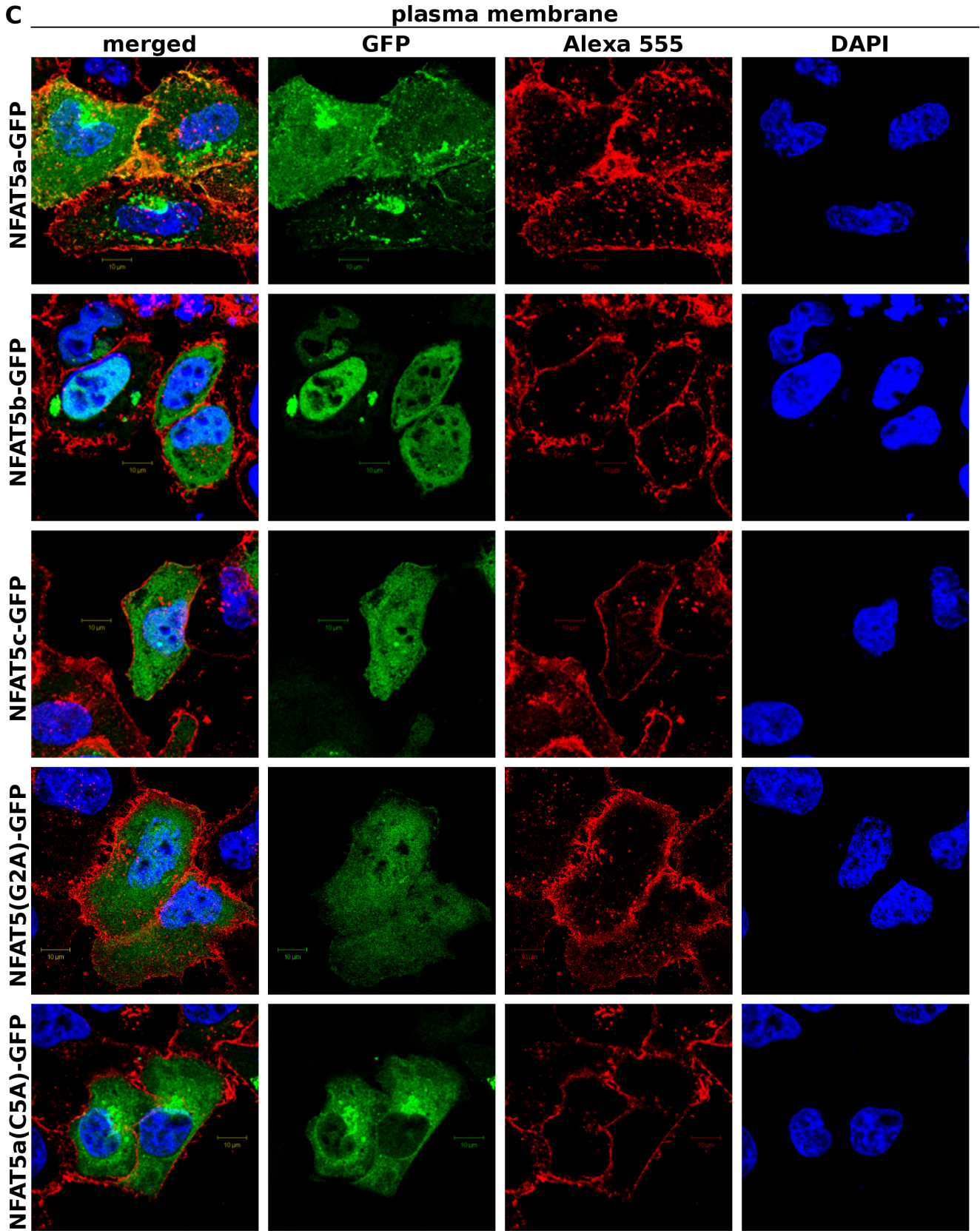
This file contains full-size images from Figure 3ABCD.

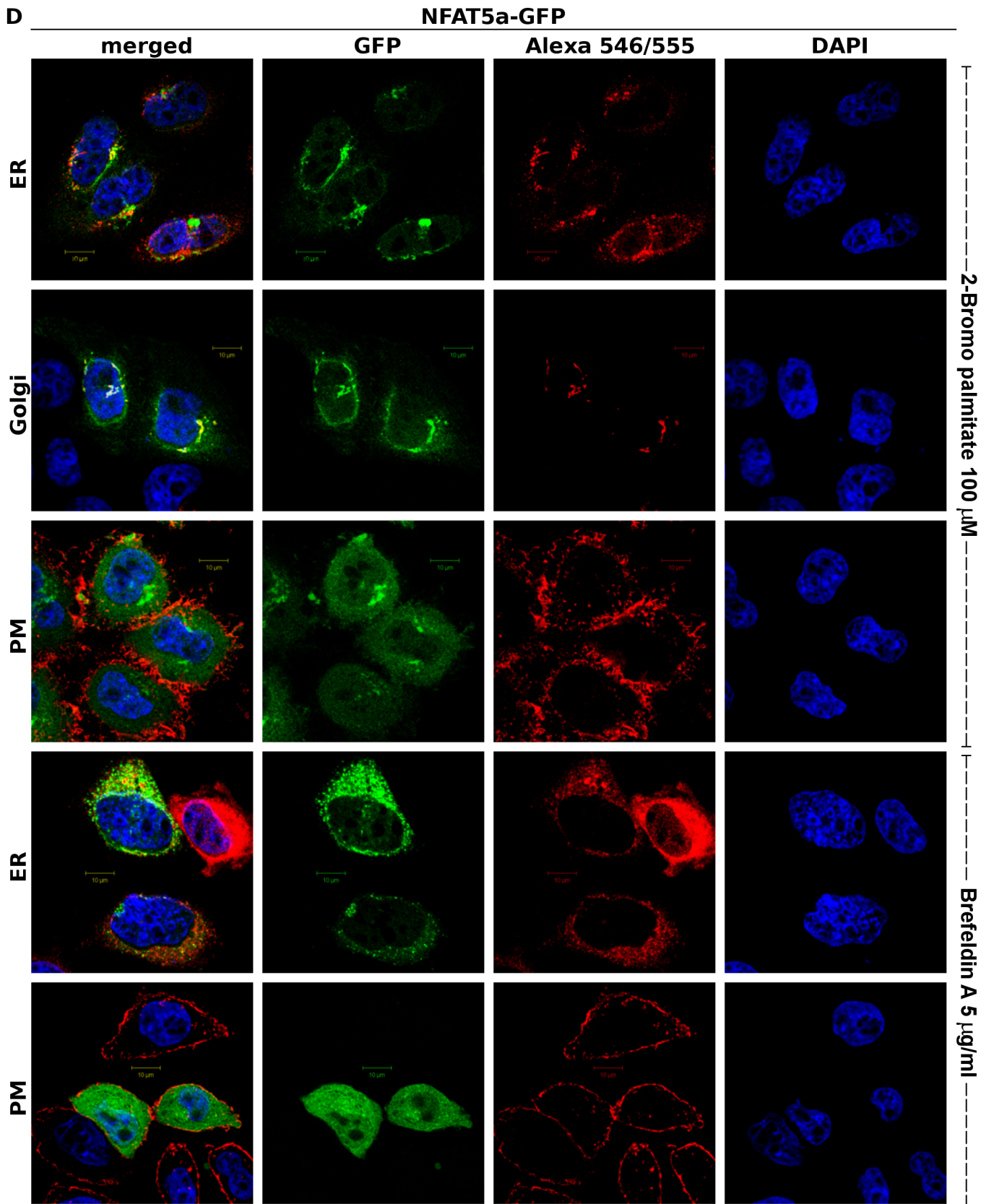
These images show the co-localization of NFAT5a/b/c-GFP and the NFAT5a mutants G2A and C5A with the ER, Golgi and the PM. NFAT5a-GFP localization in HeLa cells was also studied by competitive inhibiting palmitoylation with 2-Bromopalmitate or by disrupting the Golgi using BrefeldinA (Berthiaume et al., 1995; Draper et al., 2007).

- A. The ER was stained with anti-PDI antibodies and Alexa546 (red). NFAT5a and the C5A mutant co-localize with the ER. They show the punctuated localization pattern spread through the cell's cytoplasm like the ER. NFAT5b and c and the G2A mutant are equally distributed throughout the cell with no specific localization to the ER.
- B. Golgi was stained with Giantin and Alexa546 (red). The distinct punctuated pattern of the Golgi stain around the nucleus is clearly visible for NFAT5a and the C5A mutant. Isoforms b and c and the G2A mutant are distributed throughout the cell without specific Golgi localization.
- C. The PM was stained with wheat germ agglutinin Alexa 555 (red). NFAT5a-GFP co-localizes with the PM. None of the other constructs show PM localization to this extent.
- D. This panel shows NFAT5a transfected cells treated with two inhibitors. Under treatment with 2-Bromo palmitate, which competitively inhibits palmitoylation, NFAT5a accumulates in the ER and the Golgi, but does not co-localize with the PM. Again, both compartments can be identified by looking at the distinct pattern in the GFP channel. BrefeldinA disrupts the Golgi. NFAT5a localization to the ER can still be observed, but co-localization with the PM is inhibited.









References

- Berthiaume, L., S.M.Peseckis, and M.D.Resh. 1995. Synthesis and use of iodo-fatty acid analogs. *Methods Enzymol.* 250:454-466.
- Draper, J.M., Z.Xia, and C.D.Smith. 2007. Cellular palmitoylation and trafficking of lipidated peptides. *J. Lipid Res.* 48:1873-1884.