Full-size images for Figure 7

Anchoring of NFAT5 to the plasma membrane with the APMAP-N-terminus – full-size images

This file contains full-size images of Figure 6. This experiment is designed to establish if NFAT5a is proteolytically cleaved before nuclear import. To test this hypothesis, we attached NFAT5a/b/c to the PM using the transmembrane domain of APMAP, a constitutively PM localized protein [1]. In these conditions, it is thought that the non-globular N-terminal region should remain accessible for cleavage by a hypothetical membrane attached protease.

The images show a selected subset of confocal images of transiently transfected HeLa cells with the following GFP constructs: NFAT5a/b/c-GFP, APMAP(AA1-61)-GFP (control), APMAP(AA1-61)-NFAT5a/b/c-GFP. Salt stress (350 mM NaCl) was applied for 1 hour. Nuclei were stained with DAPI (blue). NFAT5 and APMAP constructs are GFP-labelled (green). APMAP(AA1-61) represent the transmembrane region of the APMAP protein and is constitutively localized at the PM. All NFAT5 isoforms shuttle into the nucleus during salt stress. After anchoring them to the plasma membrane using APMAP(AA1-61) none of the constructs are able to shuttle into the nucleus. This indicates that proteolytical cleavage of the N-terminus is not a likely mechanism for releasing NFAT5 from the membrane, since this region is non-globular and should be readily accessible for membrane-resident proteases.

References

1. Bogner-Strauss JG, Prokesch A, Sanchez-Cabo F et al. Reconstruction of gene association network reveals a transmembrane protein required for adipogenesis and targeted by PPARgamma. *Cell Mol Life Sci*. 2010;67:4049-4064.

Figure S7

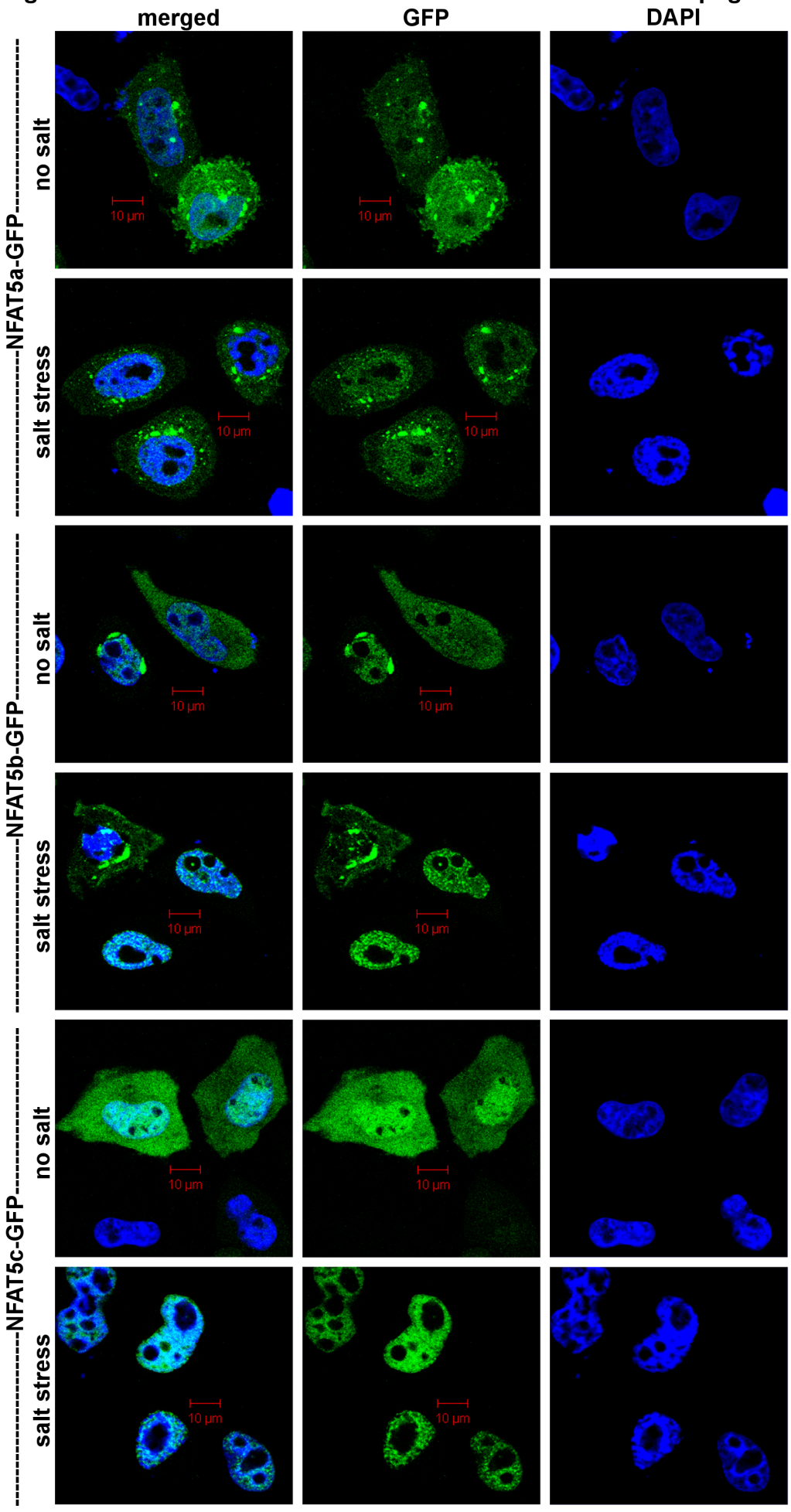

merged
GFP
DAPI
NFAT5a-GFP
no salt
salt stress
NFAT5b-GFP
no salt
salt stress
NFAT5c-GFP
no salt
salt stress
10 µm

Figure S7

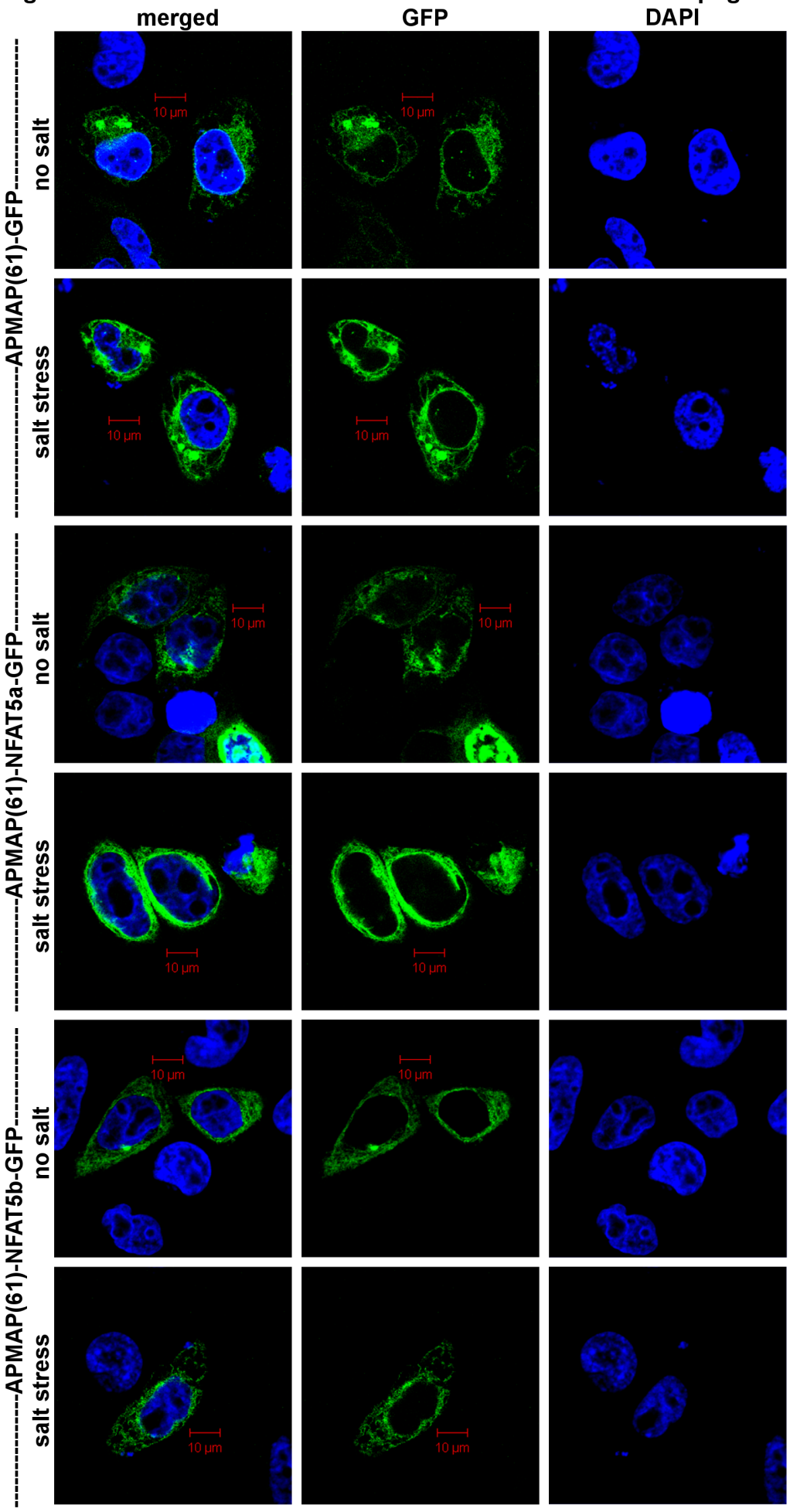
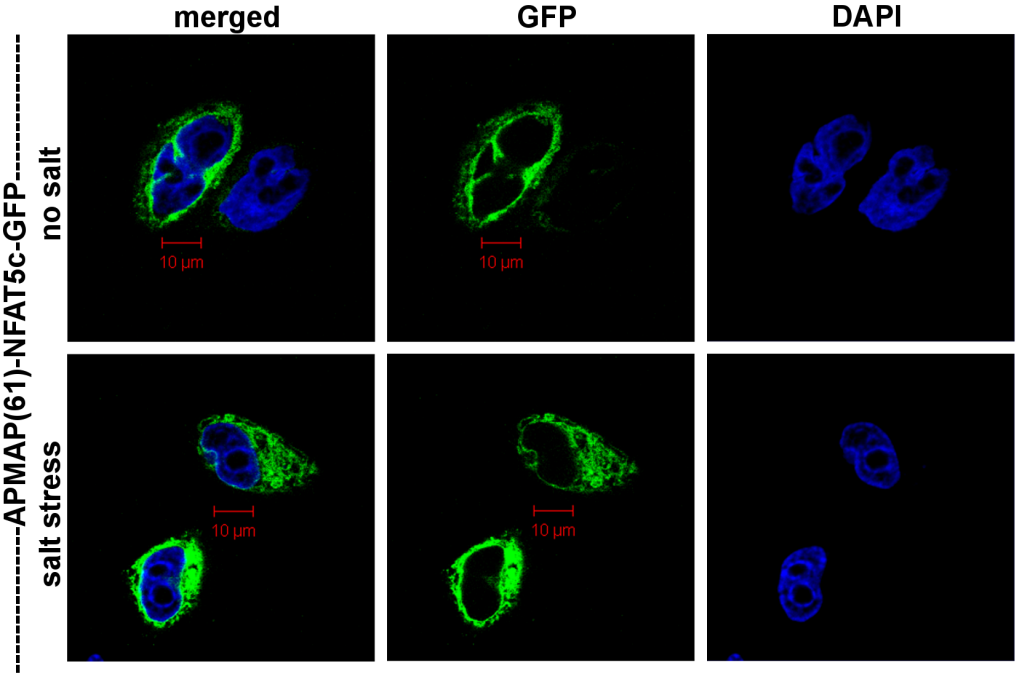

merged
GFP
DAPI
----------APMAP(61)-GFP----------
no salt
salt stress
----------APMAP(61)-NFAT5a-GFP----------
no salt
salt stress
----------APMAP(61)-NFAT5b-GFP----------
no salt
salt stress
10 µm

Figure S7


merged
GFP
DAPI
----------APMAP(61)-NFAT5c-GFP----------
no salt
salt stress
10 µm
10 µm
10 µm
10 µm