Project 2 (P2): Localization study of endogenous fluorescent protein (FP) tagged hATG8 in presence and absence of HIV-1 Nef

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The viral nef gene product is crucial for the pathogenicity of human immunodeficiency virus type 1 (HIV-1). Extracellular Nef protein that is secreted from infected cells via extracellular vesicles (EVs) triggers the extensive impairment of uninfected bystander cells during HIV-1 infection. Our aim is the elucidation of the intracellular transport and unconventional secretion pathways of Nef. We unraveled that Nef secretion relies on the availability of GABARAP, GABARAPL1 or -L2, which all belong to the GABARAPs-subfamily of human ATG8s. In this project the subcellular localization of Nef and the GABARAPs is planned to be elucidated in depth using conventional and super-resolution microscopy. Importantly, all GABARAPs are to be produced as fluorescent protein (FP) fusions under endogenous expression levels, because overexpression is an apparent concern in microscopy studies and produces numerous artifacts including ectopic sub-cellular localizations, aggregation or inaccurate protein complexes. FP-gene-knockins can be established by CRISPR/Cas9-mediated genome editing. Studying FP-GABARAPs localization in appropriate edited cell lines in combination with a simultaneous localization analysis of selected EV-subtype markers will help to unravel the mechanisms behind Nef secretion. Such knowledge is supportive for the development of effective Nef inhibitors.