Autophagy-relevant proteins LC3B and GABARAP fused to fluorescent proteins form structures of similar size but different shape

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Autophagic structures containing mammalian autophagy-related proteins GABARAP and LC3B, two members of the Atg8-protein family, have been investigated with conventional wide-field fluorescence and single molecule localization microscopy (SMLM), employing transient overexpression of both proteins as EYFP fusion proteins. On the basis of the well-known blinking of EYFP, super-resolution images of the corresponding GABARAP- and LC3B-containing structures with resolution of well below 50 nm were generated. These structures were quantitatively investigated (size, shape) for 10 representative cells and classified accordingly. While size distributions of structures labelled by the two proteins were found to be similar, shape distributions appeared quite disparate, with EYFP-GABARAP favouring circular structures and elliptical structures being dominant for EYFP-LC3B. The latter also featured a significantly enlarged fraction of U-shaped structures. The experimental results point towards highly differential localisation of the two proteins, which appear to label structures representing distinct stages or even specific channels of autophagy and vesicular trafficking pathways. Our data also demonstrate that the application of super-resolution techniques expands the possibilities of fluorescence-based methods in autophagy studies and in some cases can rectify conclusions obtained from conventional fluorescence microscopy with diffraction-limited resolution. We also performed similar experiments in cells stably transfected with Dendra2-GABARAP and Dendra2-LC3B fusion proteins, respectively, allowing photoswitching PALM super-resolution microscopy. This enabled us to compare the influence of the nature of the fluorescent protein as well as the level of overexpression on size and shape distribution of GABARAP and LC3B.