A CLOSE-UP VIEW OF MITOPHAGY USING MT-KEIMA AND SUPER-RESOLUTION MICROSCOPY

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Mitophagy is a cellular process that selectively removes damaged, old or dysfunctional mitochondria. Defective mitophagy is thought to contribute to normal aging and to various neurodegenerative and cardiovascular diseases. Previous methods used to detect mitophagy in vivo were cumbersome, insensitive and difficult to quantify. We created a transgenic mouse model that expresses the pH-dependent fluorescent protein mt-Keima in order to more readily assess mitophagy. Keima is a pH-sensitive, dual-excitation ratiometric fluorescent protein that also exhibits resistance to lysosomal proteases. At the physiological pH of the mitochondria (pH 8.0), the shorter-wavelength excitation predominates. Within the acidic lysosome (pH 4.5) after mitophagy, mt-Keima undergoes a gradual shift to longer-wavelength excitation. We describe how to apply mt-Keima with stimulated emission depletion (STED) microscopy to visualize mitophagy in various living tissues including skeletal muscle, heart (fig.1), liver, adipose tissue, and kidney, obtained from mt-Keima transgenic mice [1]. Thus, we can assess this process at nanoscale resolution (50nm) in normal living tissues but also how tissues mitophagy is altered following changes under genetic perturbations, or aging [2]. In addition, we show how to monitor mitophagic flux in living cells exploring mitophagy relationships with other cellular compartments via high resolution confocal microscopy combined with deconvolution to achieve resolution of ~ 120nm of multiple cellular compartments. In conclusion, this approach enable to explore mitochondrial dynamics and mitophagy interconnections in health and disease conditions.

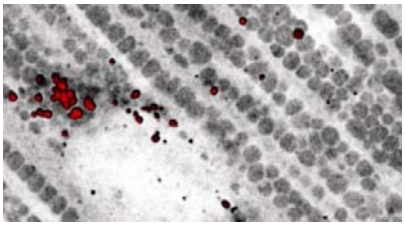

Figure 1: STED image of mt-Keima mouse heart depicting healthy mitochondria (black and white) and peri-nuclear mitophagic compartment in red.

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