Point laser scanning confocal microscopy was performed on a Leica SP8 microscope using HC PL APO CS2 63x/1.4 N.A. oil immersion objective. Live-cell imaging was performed at 37˚C and 5% CO2. Image were acquired at multiple positions using an automated stage and the Adaptive Focus Control (AFC) for focus stabilization with a time-resolution of 5 min/stack for 24h. Multichannel images were acquired sequentially using HyD detectors in the standard mode with the laser excitation at 488nm and spectral detection window set between 500 and 550nm for GFP imaging and 561nm laser excitation and spectral detection window set between 580nm and 620nm for RFP imaging. When PMT detectors were used the gain was typically adjusted between 600 and 900V, offset was typically not or minimally (≤1%) adjusted. Brightfield images were obtained from a transmitted light PMT detector. Typical sampling was 80nm in xy axis and 500nm in z axis with scanning speed between 600 and 1000Hz, bidirectionally with 2x line averaging and 8-bit digitization. Subsequently, images were processed with the Lightning algorithm in the adaptive mode using default settings.

Alternative objectives:

HC PL APO CORR CS2 63x/1.3 N.A. glycerol

HC PL APO IMM CORR CS2 20x/0.75 N.A. (with H2O, glycerol or oil)

Available laser lines:

405 nm, 458 nm, 476 nm, 488 nm, 496 nm, 514 nm, 561 nm, 633 nm