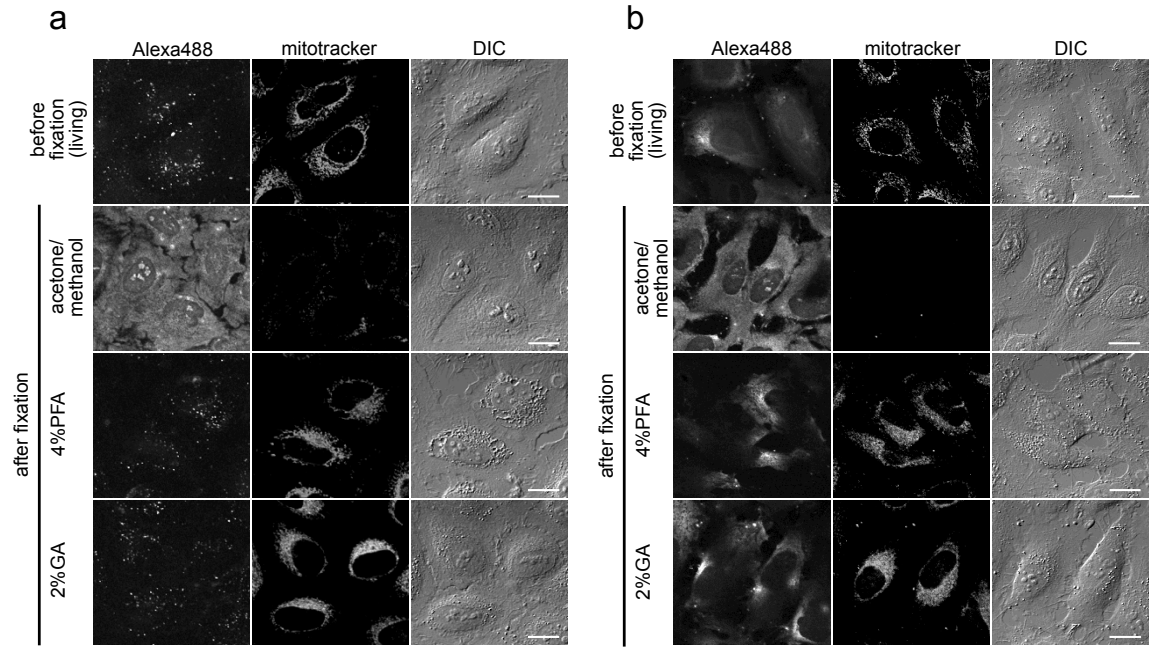


**Figure S7 Effects of fixation procedures on the internalization of R12-Alexa488 and cellular morphology.** (**a**) HeLa cells were incubated with R12-Alexa488 (5 μM) and MitoTracker (250 nM) in serum-containing medium [α-MEM(+)] for 30 minutes (i.e., conditions enabling the endocytic uptake of R12-Alexa488), washed twice with cold PBS(+), and then fixed through incubation at 4°C with acetone/methanol (1:1) for 1 minute, 4% paraformaldehyde/PBS(–) (4% PFA) for 30 minutes, or 2% glutaraldehyde in 30 mM HEPES (2% GA) for 30 minutes. After fixation, the cells were washed twice with cold PBS(+) and analyzed by confocal microscopy without using mounting medium. (**b**) Similar experiments to those described in (**a**) were conducted in serum-free medium [α-MEM(–)] (peptide treatment, 5 minutes) (i.e., conditions enabling the direct internalization of R12-Alexa488). Drastic changes in the cellular distribution of the R12 peptide, as well as that of MitoTracker, were seen in cells fixed with acetone/methanol. For cells fixed with 4% PFA or 2% GA, no significant effects were detected (note that the use of mounting media may cause artifacts in peptide localization and was avoided). These data strongly suggest that careful fixation could minimize alterations in the cellular localization of R12-Alexa488. No significant alterations in the cellular localization of R12-Alexa488 were observed in cells fixed with 4% PFA or 2% GA. Furthermore, the intracellular diffusion process following direct internalization of R12-Alexa488 can be arrested at an intermediate stage using these fixation conditions. Scale bar = 20 μm.