



Figure S1 Direct internalization of R12-Alexa488 into the cytosol, initiated at a site on the plasma membrane with an intense fluorescent signal. (a) Real-time images of cells incubated with R12-Alexa488 (5 μM) in serum-free medium [$\alpha\text{-MEM}(-)$] at 37°C. The R12 peptide was added at $t = 0$ second (see also **Video S1**). (b) Time-course analysis of fluorescence intensities at the indicated regions (1–4, cytoplasm; 5, nucleus) in the cell shown in (a) at 30-second intervals. The site of peptide influx is located in region 1. A strong fluorescent signal was observed at the influx point approximately 200 seconds after peptide administration, reaching maximum intensity approximately 450 seconds after administration. The fluorescent signals in regions 2–4 became more gradual, along with the distance from the initiation site. Scale bar = 20 μm .