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Fig. 1 Fluorescence emission spectra of probes at different pH. Representative uncorrected fluorescence emission spectra (n = 3) of pH-Her (a) and Alexa-Her (b) measured in PBS at pH of 7.5 (black curve) and of 5.5 (red dashed curve) shown as examples for conjugates with a DP ratio of 1.6 (n = 3); excitation was at λ_{ex} 635 nm.



Fig. 3 Fluorescence microscopy demonstrates internalization-dependent activation of pH-Her. Breast cancer cells grown on culture slides were incubated for 8 h with pH-Her or Alexa-Her. On the left panel, counterstain of cell nuclei with Hoechst 33342, in the middle, probe-derived signals, and on the right panel, merged images of the cell nuclei (blue) and the probe (red) are illustrated. (a), When incubated with KPL-4 cells at 37°C, pH-Her shows fluorescence only after receptor-mediated internalization (green arrow). (b), At 4°C, no signals from the pH-sensitive probe presumably bound to the cell membrane can be detected. (c), Alexa-Her shows fluorescence from the internalized probe (green arrow) and also membrane-derived fluorescence can be observed after 8 h of incubation at 37°C (c) and 4°C (d) (orange arrow, no internalization). Representative images of three independently performed experiments are presented. Bars represent 50 μ m.